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The antibiogram as an aid in the identification of the Klebsiella-enterobacter-serratia group

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THE ANTIBIOGRAM AS AN AID IN THE IDENTIFICATION OF THE KLEBSIELLA-ENTEROBACTER-SERRATIA GROUP

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I. INTRODUCTION AND HISTORICAL REVIEW

Members of the Klebsiella-Enterobacter-Serratia (K-E-S) group of the tribe Klebsielleae, family Enterobacteriaceae are widely distributed in nature and commonly found as normal inhabitants of the digestive tract of man and other vertebrates. Occasionally they are found in small numbers in or on extraintestinal areas of the body which normally harbor other bacteria. Until the introduction of modern chemotherapy, the group, with the exception of Klebsiella pneumoniae, was considered of minor significance as a cause of serious infections. Since the introduction of antibiotics, however, and along with other Gram negative bacilli, they came to assume an increasing role as cause of life-threatening infections in both hospitalized and non-hospitalized patients.

Edwards and Ewing (1972) give the following characterization of the tribe Klebsielleae. "Klebsielleae are motile or nonmotile bacteria that conform to the definition of the family Enterbacteriaceae. Hydrogen sulfide is not produced and urea is not hydrolyzed rapidly but delayed reactions may occur. With few exceptions indole is not produced, the Methyl Red test is negative, and the Voges-Proskauer reaction is positive. Growth occurs in Simmons' citrate medium and in medium containing potassium cyanide. Phenylalanine is not deaminated. Sodium alginate is utilized as a sole source of carbon by certain members of only one genus (Klebsiella), and lipase is produced by members of the genus Serratia."

The clinical assessment of the K-E-S group has been seriously compromised in the past by confusion in the taxonomy and nomenclature. Identifying and separating the "colon-aerogenes" group has been based on the characteristic appearance of colonies of the lactose-fermenting Enterobacteriaceae on eosin methylene blue agar (EMB). This readily distingui-

shes typical Escherichia coli from the Klebsiella-Enterobacter-Serratia group with a certain degree of accuracy, but fails to identify atypical strains, and encourages the reporting of these organisms as "coliforms" or K-E-S. The suggestion of Thaler (1962) that infections by these different organisms are similar and require similar treatment is no longer acceptable. The introduction of new antibiotics and chemotherapeutic agents such as ampicillin, cephalosporins, carbenicillin and nitrofurantoin, with a wide range of effectiveness against the K-E-S group and other Gram negative bacteria requires precise identification of the infectious agents to institute proper chemotherapy.

It is beyond the scope of this work to list all the names and studies of the numerous investigators who have contributed to our current knowledge of the taxonomy of the group. The names of Edwards and Ewing should be mentioned, however. Through a series of papers and monographs, starting with Edwards' publications in the late 1920's and jointly with Ewing in the mid-1950's and culminating in their classical monograph of 1972, and later by Ewing and Fife (1972) and Ewing et al. (1973), the Enterobacteriaceae became well known and characterized. In this paper the author adheres to the taxonomic system based on their studies and accepts their recognition of ten species in three genera: Klebsiella with three species: K. pneumoniae, K. ozaenae, K. rhinoschleromatis; Enterobacter (formerly Aerobacter) with four species: E. cloacae, E. aerogenes, E. hafniae, E. agglomerans; and Serratia with three species: S. marcescens, S. liquefaciens, and S. rubidaea.

The identification of members of the K-E-S group has been facilitated in recent years through the introduction of various commercial kits, including the Enterotube (Hoffmann-La Roche), API 20 (Analytab Products), AuxoTab (Colab Laboratories), PathoTec (General Diagnostics), and R/B

tubes (Diagnostic Research Inc.). The expense involved in using these kits for complete identification (about $2.00 per kit) prohibits their routine use, and most laboratories resort to the identification of these organisms through the use of a few tests which quite often do not lead to a complete and definitive identification to the species level. A number of investigators have observed and suggested that certain patterns of antibiotic susceptibility testing might be used empirically in the identification of these bacteria and in conjunction with a few biochemical tests contribute to a more definitive identification.

Herrell et al. (1964) studied 120 strains of Klebsiella from various clinical sources over a one year period. Eickhoff et al. (1966) studied 306 strains of the Klebsiella-Enterobacter (Aerobacter) K-E(A) group and compared their biochemical and serologic characteristics with susceptibility to 16 antibiotics. Koch and Rose (1966) investigated 75 strains of the K-E(A)-S division and compared their reactions to cephalothin and ampicillin. Lerner and Weinstein (1967) reported on differentiation of Klebsiella from Enterobacter (Aerobacter) species by sensitivity to cephalothins and penicillins. Edmondson and Sanford (1967) evaluated 184 strains of the Klebsiella-Enterobacter-Serratia group with regard to clinical and bacteriological characteristics. Ramirez (1968) studied the differentiation of 67 strains of Klebsiella-Enterobacter-(Aerobacter)-Serratia by biochemical tests and compared their antibiotic susceptibilities. Zabransky et al. (1969) compared the biochemical characteristics and susceptibility of 329 strains to a selected group of antibiotics under clinical conditions. Washington and Bourgeois (1969) investigated the discrepancies between the disc diffusion and the tube dilution methods of susceptibility of 125 strains of Klebsiella-Enterobacter to cephalothin.

Washington et al. (1969) studied the biochemical activity and antibiotic susceptibility of 52 strains of atypical Enterobacter cloacae isolated over a six-month period. Russell (1969) determined antibiotic susceptibilities of 478 strains of Klebsiella-Enterobacter isolates from clinical infections. Wilfert et al. (1970) studied the incidence and antibiotic susceptibilities of Serratia marcescens. Greenup and Blazevic (1971) investigated the similarities in antibiograms of Serratia marcescens and Enterobacter (Serratia) liquefaciens. Friedman and MacLowry (1973) described a computer program utilizing a Baysean mathematical model to identify bacteria solely on the basis of their antibiotic sensitivities; and more recently Darland (1975) reported on antibiotic susceptibility patterns as an effective tool in the identification of bacteria. Klein et al. (1975) investigated the relationship of indole production of 250 strains of K. pneumoniae to antibiotic susceptibility.

Some of the results of the earlier studies (chiefly those prior to 1972) have been conflicting, in part because of the taxonomic problems. These studies also utilized the time consuming Minimal Inhibitory Concentration (MIC) technique which has recently been replaced in many clinical laboratories by the Bauer-Kirby (B-K) disc diffusion method.

The purpose of this study was to examine and evaluate the K-E-S group from this community with respect to distribution in clinical materials, detailed individual biochemical characteristics, and antibiogram patterns using the Bauer-Kirby disc technique, and compare these findings with those of other investigators from other geographic locations. As far as can be determined, this is the only study of its kind on the West Coast of the United States.

II. MATERIALS AND METHODS

Two hundred and three strains of the K-E-S group were obtained from various clinical materials (Table I) from in-and-out patients at Dameron Hospital Laboratory, Stockton, California, between January 1974 and February 1976. The organisms were subcultured on eosin-methylene blue agar (EMB) plates to ascertain their purity, and individual colonies streaked on tryptic soy agar (TSA) slants to serve as stock cultures. All stock cultures were stored at 2-8° C for later studies. The reactions of the isolates in 24-27 selected enzymatic, fermentative and motility tests (Tables II, III, IV) were then determined and strains identified by the methods of Edwards and Ewing (1972). No single biochemical test was given any unusual weight except for strains assigned to the genus Klebsiella, which, by definition are non-motile. Incubation temperature was set at 35° C, except for those non-motile strains that were not clearly K. pneumoniae or K. ozaenae. These were also tested for motility at room temperature.

The following tests were utilized to investigate the biochemical activities of the isolates: indole production (tryptone), acidity (Methyl Red), acetoin production (Voges-Proskauer), citrate utilization (Simmons'), hydrogen sulfide production (TSI), urease activity (Christensen's) motility (stab), decarboxylation of lysine and ornithine, dehydrolation of arginine, fermentation and gas production from glucose, inositol and sorbitol, fermentation of lactose, sucrose, mannitol, dulcitol, salicin, adonitol, arabinose, raffinose, rhamnose, melibiose and xylose, utilization of malonate, deoxyribonuclease activity and pigment production. Following the identification of each strain, its reaction to eleven antibiotics (Table V) was examined utilizing the Bauer-Kirby agar disc-diffusion tech-

nique on 150 mm Mueller-Hinton plates and standardized conditions as recommended by Bauer et al. (1972). Except for the motility tubes and Christensen's Urea agar slants, which were purchased from Microbiological Media, Concord, California, all other media were prepared by the author. In addition to the above media, the Enterotube (Hoffmann-La Roche) and its numerical coding and identification system (Encise II) were utilized to identify some strains. The API 20 (Analytab Products) was also used to determine the identity of two strains. The antibiotic discs used were Difco products (Difco, Detroit); their potency and zone interpretation are indicated in Table V.

Procedures used to run these tests are given on pages 80-86.

III. KLEBSIELLA

Edwards and Ewing (1972) characterized the genus Klebsiella as follows: "The genus Klebsiella is composed of nonmotile bacteria that conform to the definitions of the family Enterobacteriaceae and the tribe Klebsielleae. The Voges-Proskauer test is positive; gelatin is not liquefied. Lysine decarboxylase is produced, but arginine dehydrolase and ornithine decarboxylase are not. The majority of cultures utilize sodium alginate as a sole source of carbon and esculin is hydrolyzed. Gas is formed from inositol and glycerol, and by the majority of strains from adonitol. Acid is produced from sorbitol, rhamnose, arabinose, and raffinose. The type species is Klebsiella pneumoniae (Schroeter) Trevisan."

Of the 121 strains encountered in this study in the genus Klebsiella, 120 (99.2%) strains are identified as K. pneumoniae (Friedlander's bacillus) and only one (0.8%) as K. ozaenae. The distribution of these strains in clinical material is shown in Tables I and IX. K. pneumoniae is by far the most common and most frequently encountered member of the genus. K. ozaenae is usually found in ozaena and other chronic diseases of the nasal mucosa. K. rhinoschleromatis, rare in the United States, is found constantly and exclusively in patients with rhinoscleroma and their contacts. The three species may be differentiated on the basis of twelve biochemical tests (Table VIII).

Klebsiella pneumoniae (Schroeter) Trevisan, 1887

Synonyms: *Klebsiella aerogenes*; *Klebsiella oxytocum*; *Bacterium pneumoniae crouposae*; *Hyalococcus pneumoniae*; *Bacillus pneumoniae*

DISTRIBUTION

Of 120 strains assigned to this species, 34.8% were obtained from urine, 33.9% from the respiratory tract, 15% wounds, 5.1% fecal, 2.6% blood and 9.4% others (Table IX). This compares well with Klein et al. (1975), who reported 35.6% from urine and 34.4% from the respiratory tract; other sources compare equally well. Herrell et al. (1964), Eickhoff et al. (1966), and Edmondson and Sanford (1967), reported a higher incidence of isolation from urine: 52.5%, 59.3% and 62.5%, respectively. Herrell et al. (1964) and Edmondson and Sanford (1967) also reported the respiratory tract as being the next common source with 31.7% and 19.6%, respectively; Eickhoff et al. (1966) found 19.8% of the isolates in blood, but only 8.6% from the respiratory tract. In our study 2.5% of the strains were from blood. The 18 wound isolates included six burns, five incisions, and three abcesses.

It is of interest to note the difference between the findings of Eickhoff and his group and those of Lerner and Weinstein. Both dealt with isolates from the same geographic location (Boston) but from two different hospitals, Boston General and the New England Medical Center respectively. Lerner and Weinstein (1967) make no reference to Eickhoff et al. (1966).

BIOCHEMICAL TESTS

The biochemical activities of *K. pneumoniae* strains obtained in this study are compared with those of Edwards and Ewing (1972) and are shown in Table II. All *K. pneumoniae* strains were hydrogen sulfide and DNase negative, failed to decarboxylate ornithine, and fermented glucose, lactose, sucrose, mannitol, sorbitol, arabinose, raffinose and rhamnose. Over 90% of the strains gave a positive reaction in the Voges-Proskauer test, utilized citrate, produced urease, decarboxylated lysine, produced gas from glucose, fermented salicin, adonitol and inositol, and utilized malonate. Over 90% of the strains were negative for indole production and arginine dehydrolysis. Only 11.7% of the strains were Methyl Red positive, and fermentation of dulcitol was variable (33.3% positive).

ANTIBIOTIC SUSCEPTIBILITIES

Except for one strain which was susceptible to all antibiotics used, the others exhibited various degrees of susceptibility and resistence (Figure I).

Over 90% of the strains were resistant to carbenicillin and susceptible to cephalothin, polymyxin B, gentamycin, kanamycin and neomycin. Resistance to ampicillin was evident in 88% of the strains. Reaction to the other antimicrobial drugs was variable.

The antibiotic patterns of indole positive and indole negative strains are shown in Figures II and III, respectively.

Two techniques are normally used by inwestigators to study the in-vitro effect of antimicrobial substances on microorganisms; the Bauer-Kirby disc diffusion technique (Procedures on page 85), and the Minimum Inhibitory Concentration (MIC) procedure which is considered to be more accurate, but more time consuming. The following description has been

adopted from Lennette and Spaulding.(1974): "Dilution tests are used to determine the minimal concentration of an antimicrobal agent required to inhibit or kill a microorganism. Serial dilutions of the antimicrobal agent are inoculated with the organism and incubated. The minimum inhibitory concentration (MIC) is the lowest concentration without apparent growth. The term 'broth' (or 'tube') and 'agar' (or 'plate') is added to the term 'dilution test,' depending on whether the test is performed in liquid or agar media, respectively. Both terms are actually misnomers because it is the antimicrobial agent that is being diluted rather than the broth or agar."

Susceptibility studies were conducted by a number of investigators including Herrell et al. (1964), Eickhoff et al. (1966), Koch and Rose (1966), Lerner and Weinstein (1967), Edmondson and Sanford (1967), Ramirez (1968), Sanford (1969), Washington and Bourgeois (1969), Zabransky et al. (1969), Russell (1969) and Klein et al. (1975). Some of these investigators used the Minimum Inhibitory Concentration (MIC) technique, dilution depending on antimicrobial substance used; others used the disc diffusion method. A few reported their findings on the basis of species, but the majority at the generic level. Some used as few as two antibiotics, others as many as 16. It should also be indicated that differences in sensitivity patterns among various studies may be due to testing methods, degree of usage of a particular antimicrobial substance in the community, or the carrier rate of resistant bacteria by hospital personnel. Comparisons between their studies and the present one are, therefore, difficult to interpret but some general conclusions and remarks may be relevant and appropriate.

Ampicillin

Twelve per cent of the strains in the study were found sensitive to ampicillin. Other investigators have reported susceptibilities that varied from 1% - 17%. Using the MIC technique and concentration up to 20 ug/ml, Eickhoff et al. (1966) reported 1% sensitive, Koch and Rose (1966) 8%, Lerner and Weinstein (1967) 4.7%, Edmondson and Sanford (1967) 16%, and Zabransky et al. (1969) 17%. Klein et al. (1975), used the Bauer-Kirby (B-K) disc technique and reported 8% susceptibility.

Carbenicillin

This is a relatively new member of the penicillin group and was investigated only by Klein et al. (1975) who reported 7.5% sensitivity in comparison with the present study of 2.7%.

Cephalothin

Ninety three per cent of the strains were sensitive to cephalothin. Koch and Rose (1966) reported 100% sensitivity. Ramirez (1968) 96.9%, Washington and Bourgeois (1969) 93.8%, and Klein et al. (1975) 90% sensitive, all using B-K disc diffusion methods. On the basis of the MIC method, Eickhoff et al. (1966) reported 86% sensitivity, Koch and Rose (1966) 96%, Lerner and Weinstein (1967) 85.9%, Edmondson and Sanford (1967) 72%, Washington and Bourgeois (1969) 80%, Zabransky et al. (1969) 89%, and Russell (1969) 80%.

Polymyxin B

Ninety nine per cent of the strains were sensitive to polymyxin B. This compares well with most previous investigators. Herrell et al. (1964) reported 93% susceptibility and Eickhoff et al. (1966) 62%, both used MIC levels of 12.5 ug/ml. Edmondson and Sanford (1967)

found 89% susceptibile, with MIC methods and levels of 20 ug/ml. Klein et al. (1975) reported 100% sensitivity, with the B-K method. It is obvious that Eickhoff's findings are low.

Nitrofurantoin

Forty nine per cent of the strains were sensitive to nitrofurantoin. Edmondson and Sanford (1967) found nitrofurantoin ineffective at low concentrations (2.5 ug/ml), but uniformly effective at high concentrations (100 ug/ml) normally achieved in urine. Klein et al. (1975) found significant differences in susceptibility between indole negative (73% sensitive) and indole positive strains (100% sensitive).

Tetracycline

Seventy seven per cent of the strains were sensitive to tetracycline. This is higher than those of previous studies. Eickhoff et al. (1966) reported 23% susceptible, Edmondson and Sanford (1967) 47%, and Russell (1969) 49%; all used MIC methods with concentration levels of 12.5 ug/ml, 20 ug/ml, and 6.3 ug/ml respectively. Klein et al. (1975) found 70% of indole negative strains to be sensitive and 94% sensitivity by the indole positive strains.

Gentamycin

Ninety nine per cent of the strains were sensitive to gontamycin. Eickhoff et al. (1966) reported 90% susceptibility, Edmondson and Sanford (1967) 100%, and Russell (1969) 99%; all used MIC methods with concentration levels of 3.1 ug/ml, 5 ug/ml, and 6.3 ug/ml respectively. Klein et al. (1975) reported 100% sensitivity with the B-K method.

Kanamycin

Ninety seven per cent of the strains were sensitive to kanamycin. This is slightly higher than previous studies; Herrell et al. (1964) reported 87% susceptibility, Eickhoff et al. (1966) 84%, Edmondson and Sanford (1967) 91%, and Russell (1969) 94%, all used MIC techniques with concentration levels of 3.125 ug/ml, 12.5 ug/ml, 20 ug/ml, and 25 ug/ml respectively. Klein et al. (1975) found 83% of indole negative and 90% of the indole positive strains sensitive.

Streptomycin

Seventy six per cent of the strains were sensitive to streptomycin. This is higher than those of other studies except for Klein et al. (1975). Herrell et al. (1964) reported 37% susceptible, Eickhoff et al. (1966) 21%, Edmondson and Sanford (1967) 53%, and Russell (1969) 69%, all used MIC techniques with concentration levels of 3.125 ug/ml, 12.5 ug/ml, 20 ug/ml, and 25 ug/ml, respectively. Klein et al. (1975) found 77% of indole negative and 89% of indole positive strains sensitive.

Neomycin

Ninety eight per cent of the strains were sensitive to neomycin. This is higher than reported in previous studies. Eickhoff et al. (1966) reported 80% susceptibility using MIC techniques with a concentration level of 12.5 ug/ml. Klein et al. (1975) found 82% of indole negative and 98% of indole positive strains sensitive.

Nalidixic acid

Ninety per cent of the strains were sensitive to nalidixic acid. Klein et al. (1975) found 89% of indole negative and 98% of indole

positive strains sensitive.

Indole Reaction and Susceptibility to Antibiotics

Klebsiella pneumoniae is typically indole-negative, but a number of investigators have observed that the incidence of indole-positive strains was increasing. The ability to produce indole depends on the presence of the enzyme tryptophanase which hydrolyzes tryptone into indole, pyruvic acid and ammonia. The gene that controls tryptophanase synthesis is believed to be acquired by genetic transfer through transformation, conjugation or transduction.

Fife et al. (1965) reported that six per cent of Klebsiella strains isolated from patients between 1948 and 1964 were indole-positive. A similar incidence was reported by Eickhoff et al. (1966). More recently Martin et al. (1971) reported an incidence of 16.9% in a three-month study at Mayo Clinic, Rochester, Minnesota; Davis and Matsen (1974) found indole produced by one-third of their isolates, and Klein et al. (1975) reported 18.3% incidence among 2442 isolates obtained between 1966-1972 from University of Minnesota Hospital patients. This apparent increase of indole-positive Klebsiella strains prompted a number of investigators to explore a possible difference in antibiogram patterns of indole-positive and indole-negative strains of Klebsiella pneumoniae. Zabransky et al. (1969) found three per cent indole-positive strains among their isolates and reported no difference in susceptibility to ampicillin between the two groups. Martin et al. (1971) reported that the indole-positive strains were more susceptible to tetracycline, streptomycin, chloramphenicol, cephalothin, ampicillin, nalidixic acid and nitrofurantoin, but not to

gentamycin, kanamycin or polymyxin. The most extensive study was done by Klein et al. (1975). These authors applied the binomial test to determine significance of their data, and their results indicated "a greater incidence of multiple drug resistance among the indole-negative strains than among those that produced indole. The organisms in the former group, in comparison to their indole-positive counterparts, were significantly more resistant to nitrofurantoin, tetracycline, chloramphenicol, neomycin, streptomycin, nalidixic acid and kanamycin. Both groups of organisms were similar in the degree of resistance to ampicillin, carbenicillin, cephalothin, sulfisoxazole, colistimethate, polymyxin B and gentamycin."

In this study, seven strains (6%) were indole-positive. The small size of the sample does not lend itself properly to statistical analysis but Figures II and III suggest some differences. The indole-positive group shows greater susceptibility to nitrofurantoin, tetracycline, and streptomycin, but greater resistance to ampicillin, carbenicillin, cephalothin, kanamycin and neomycin. Susceptibility to nalidixic acid, gentamycin and polymyxin B is about the same. The extent of multiple drug resistance of indole-positive and indole-negative strains is shown in Table X.

The results of the present study agree partially with those of Martin et al. (1971) and Klein et al. (1975). Indole-positive strains seem to show greater susceptibility to tetracycline, streptomycin and nitrofurantoin and possibly chloramphenicol (not investigated in this study); our results, however, indicate decreased sensitivity to cephalothin, ampicillin, kanamycin and neomycin; no difference in susceptibility to nalidixic acid is noted, but both Martin et al. (1971)

and Klein et al. (1975) report increased sensitivity. Klein and his group observed that prolonged storage of stock cultures increases susceptibility to antibiotics but also the ability to produce indole may be lost. The transfer of genetic material by conjugation, transformation and transduction is certainly a dynamic factor in the transmission of resistance between strains; apparently it is a transient phenomenon which may be lost by cold storage. The author considers the relationship of indole production to antibiotic sensitivity a problem that requires further investigations.

Klebsiella ozaenae (Abel) Bergey, Breed, and Murray, 1925

Synonyms: Bacillus mucosus ozaenae; Bacillus ozaenae; Bacterium ozaenae

DISTRIBUTION

Only a single isolate was obtained and the site of infection was the respiratory tract.

BIOCHEMICAL TESTS

The biochemical activities of the single strain identified as K. ozaenae are shown in Table II. Characteristics useful in the identification of this species include negative indole, Voges-Proskauer, hydrogen sulfide, and DNase. Dulcitol was not fermented and malonate not utilized. Ninety per cent or more of the strains produced acidity in the Methyl Red test, fermented glucose, mannitol, salicin, adonitol, arabinose, raffinose and xylose. The other characteristics were variable.

ANTIBIOTIC SUSCEPTIBILITIES

The antibiotic susceptibility for K. ozaenae is shown in Figure IV. The results indicate resistance to ampicillin and carbenicillin and susceptibility to all others.

This organism is rarely reported in the literature, either because it is scarce or because it is missed or erroneously identified as K. pneumoniae or possibly a non-motile mutant Enterobacter or Serratia species.

No further comments or discussions will be made because of the unavailability of sufficient data on antibiotics and distribution.

IV. ENTEROBACTER

Edwards and Ewing (1972) characterize the genus Enterobacter as follows: "The genus Enterobacter is composed of motile bacteria that conform to the definitions of the family Enterobacteriaceae and the tribe Klebsielleae. The Voges-Proskauer reaction is positive; gelatin is liquified slowly by the most commonly occurring form Enterobacter cloacae . Lysine decarboxylase is not produced by E. cloacae or E. agglomerans, but other species of the genus possess this enzyme system. Ornithine decarboxylase is produced by all strains except E. agglomerans. Sodium alginate is not utilized as a sole source of carbon. Gas is not formed from inositol or glycerol by cultures of E. cloacae. Acid is produced from sorbitol, rhamnose, arabinose and raffinose by the majority of the species. One species (E. hafniae) does not ferment sorbitol or raffinose. The type species is Enterobacter cloacae (Jordan) Hormaeche and Edwards.

Of the 55 strains assigned to this genus, 39 (70.9%) were identified as E. cloacae, 15 (27.3%) as E. aerogenes, and one (1.8%) as E. agglomerans. Enterobacter hafniae was not found in this investigation.

The distribution of these species is shown in Tables I, XI, XIII, and XIV. Enterobacter cloacae is the most common, followed by E. aerogenes and E. agglomerans; E. hafniae is rare. The four species are differentiated by 13 biochemical tests (Table XII).

Enterobacter cloacae (Jordan) Hormaeche and Edwards, 1960

Synonyms: *Bacillus cloacae*; *Bacterium cloacae*; *Cloaca cloacae*; *Aerobacter cloacae*; *Aerobacter* A; *Cloaca* A

DISTRIBUTION

Of 39 strains assigned to this species, 51.3% were isolated from the respiratory tract, 30.8% from wounds, 10.3% urine, and 7.8% others. These findings do not agree with those of other investigators (Table XIII). No previous investigator reported the respiratory tract as the predominant isolation source. Most of them list the urinary tract as the predominant isolation source, followed by either the respiratory tract or the wound-abscess-incision group. Eickhoff et al. (1966) reported blood as the predominant source (46.7%). The twelve wound isolates included three from incision drainage, three from abscesses, and one from blood.

BIOCHEMICAL TESTS

The biochemical activities of *E. cloacae* strains are shown in Table III. All *E. cloacae* strains were indole, hydrogen sulfide, and DNase negative, failed to decarboxylate lysine, Voges-Proskauer positive, utilized citrate, decarboxlyated ornithine, fermented and produced gas from glucose, and fermented sucrose, arabinose and raffinose. Over 90% of the strains were positive for arginine hydroylsis and fermented mannitol and sorbitol. Over 90% of the strains gave a negative Methyl Red test. Variable results were obtained for urease production (72.5%), fermentation of lactose (76.9%), dulcitol (15.4%), salicin (74.4%),

adonitol (30.8%), inositol (17.9%) and rhamnose (84.6%) and utilization of malonate (84.6%). Comparing these findings with the figures in parenthesis reveals excellent correlation.

Although motility is a characteristic of the genera Enterobacter and Serratia but not Klebsiella, a certain number of strains identified by detailed biochemical tests proved to be Enterobacter or Serratia. Among E. cloacae two strains (5.1%) were non motile.

ANTIBIOTIC SUSCEPTIBILITIES

The reaction of E. cloaeae strains to the eleven antibiotics is shown in Figure V.

All of the strains were resistant to cephalothin, and sensitive to polymyxin B, gentamycin, kanamycin, neomycin, and nalidixic acid. Over 90% of the strains were sensitive to streptomycin. Resistance to ampicillin was evident in 90% and to carbenicillin in 82%. Reactions to the other antimicrobial drugs were variable.

Ampicillin

Ten per cent of the strains were sensitive to ampicillin. Other investigators have reported susceptibility ranging from 0 to 28%. Koch and Rose (1966) reported 24% susceptible; Edmondson and Sanford (1967) and Washington et al. (1969) 100% resistance. Koch and Rose (1966) used the MIC technique with concentration levels of 6.5 ug/ml and Edmondson and Sanford (1966) and Washington et al. (1969) 10 ug/ml. Koch and Rose (1966) also reported 28% susceptibility using an agar disc diffusion method.

Carbenicillin

Eighty two per cent of the strains were sensitive to carbenicillin. As indicated earlier this is a relatively new member of the penicillin group and is here investigated for the first time on a comparative basis.

Cephalothin

All of the strains were resistant to cephalothin. This compares well with the findings of other investigators. Koch and Rose (1966) reported 8% susceptible; Lerner and Weinstein (1967), Edmondson and Sanford (1967), and Washington and Bourgeois (1969) 100% resistance; and Washington et al. (1969) 8% susceptibility, all using the MIC technique and concentration levels of 10 ug/ml, 20 ug/ml, 10 ug/ml, 12.5 ug/ml, and 10 ug/ml, respectively. Koch and Rose (1966) reported 8% susceptible using an agar disc diffusion method, with susceptibility or resistance based on the presence or absence of a zone of inhibition regardless of size. Washington and Bourgeois (1969) reported 7.1% susceptible using a modification of the disc diffusion method.

All species of Enterobacter are capable of producine the enzyme cephalosporinase, which in all likelihood, is an inducible enzyme as evident by the fact that a species or strain may be initially sensitive but will develop resistance in time to the cephalosporins.

Nitrofurantoin

Sixty two per cent of the strains were sensitive to nitrofurantoin. Edmondson and Sanford (1967) found nitrofurantoin ineffective at low concentrations (2.5 ug/ml), but uniformly effective at high concentrations (100 ug/ml) as achieved in urine.

Polymyxin B

All of the strains were sensitive to polymyxin B. Edmondson and Sanford (1967) reported 73% inhibition, and Washington et. al (1969) 98%, both using the MIC technique and concentration levels of 10 ug/ml.

Tetracycline

Seventy seven per cent of the strains were sensitive to tetracycline. Previous investigators differ greatly in their results. Eichkoff et. al (1966) reported 40% susceptible and Washington et. al (1969) 94%; both used MIC techniques with concentration levels of 20 ug/ml and 10 ug/ml, respectively.

Gentamycin

All of the strains were sensitive to gentamycin. This is in agreement with Edmondson and Sanford (1967) who also reported 100% inhibition.

Kanamycin

All of the strains were sensitive to kanamycin. This compares well with Edmondson and Sanford (1967) who reported 94%, and Washington et. al (1969) 100% susceptibility, both using the MIC technique and concentration level of 10 ug/ml.

Streptomycin

Ninety two per cent of the strains were sensitive to streptomycin, in contrast with the previously reported 55% inhibition by Edmondson and Sanford (1967). Streptomycin is not as often used today as in previous years. It is quite possible that the high per cent of resistance to streptomycin indicated by Edmondson and Sanford (1967) is a reflection of the common use of this antibiotic in the 1960's which contributed to the

development of resistance by a number of bacterial species. Streptomycin is not commonly used anymore in the Stockton community because of its many undesirable side effects.

Neomycin

All of the strains were sensitive to neomycin. Neomycin has a very similar antibiotic spectrum as Kanamycin; both are aminoglycosides to which most bacteria, if not all, exhibit the same degree of reaction. Most investigators test one or the other and rarely both.

Nalidixic acid

All of the strains were sensitive to nalidixic acid. Greenup and Blazevic (1971) reported 100% susceptibility using both the B-K method, and the MIC technique with a concentration level of 12.5 ug/ml.

Enterobacter aerogenes (Kruse) Hormaeche and Edwards 1960

Synonyms: Bacillus aerogenes; Aerobacter aerogenes; Aerobacter B; Cloaca B

DISTRIBUTION

Of 15 strains assigned to this species, 53.3% were obtained from the respiratory tract, 26.7% from urine, and 20% from wounds (Table I and Table XV). Our study indicates a much higher incidence of respiratory tract infections or colonization and a lower number of urinary isolates. Eickhoff et. al (1966) reported 68.8% of their isolates from urine, 18.8% from the respiratory tract, and 12.5% from blood. Edmondson and Sanford (1967) reported 50% of the isolates from urine, 37.5% from the respiratory tract, and 6.3% from blood. This is possibly a change in epidemiologic pattern of infections by this organism or a reflection of local geographical differences.

BIOCHEMICAL TESTS

The biochemical activities of the E. aerogenes strains are shown in Table III. All E. aerogenes strains were hydrogen sulfide and DNase negative, failed to dehydrolate arginine, utilized citrate, decarboxylated lysine and ornithine, utilized malonate, fermented and produced gas from glucose and inositol, and fermented lactose, sucrose, mannitol, salicin, adonitol, arabinose, raffinose and rhamnose. Over 90% of the strains gave a positive reaction in the Voges-Proskauer Test. Over 90% of the strains gave a negative reaction in the Methyl Red Test. Indole and urease production and the fermentation of dulcitol were variable (33.3% positive). Comparing these findings with those figures in parenthesis (Edwards and Ewing 1972) reveals good correlation.

Among the strains of E. aerogenes four (26.7%) were non motile.

ANTIBIOTIC SUSCEPTIBILITIES

Except for one strain which was susceptible to all, the others exhibited varying degrees of susceptibility or resistance (Fig. VI).

All of the strains were sensitive to polymyxin B, gentamycin and kanamycin. Over 90% of the strains were sensitive to neomycin and nalidixic acid; and resistant to cephalothin. Resistance to ampicillin was evident in 87% of the strains. Reaction to the other antibiotics were variable.

Ampicillin

Thirteen per cent of the strains were sensitive to ampicillin. Edmondson and Sanford (1967) reported 22% inhibition and Lerner and Weinstein (1967) 17%, both used the MIC technique with a concentration levels of 20 ug/ml and 12 ug/ml, respectively.

Carbenicillin

Sixty seven per cent of the strains were sensitive to carbenicillin.

Cephalothin

Seven per cent of the strains were sensitive to cephalothin. This agrees with the findings of other investigators. Edmondson and Sanford (1967) reported cephalothin to be ineffective; Lerner and Weinstein (1967) and Washington and Bourgeois (1969) reported 100% resistance; all used the MIC technique with concentration levels of 10 ug/ml and 12.5, 12 ug/ml, respectively. Washington and Bourgeois (1969), however, also found 31% sensitivity with the B-K disc method on the same strains tested with the MIC technique. It is generally believed that the MIC technique, although

more time consuming to perform, is more accurate.

Polymyxin B

All of the strains were sensitive to polymyxin B. Edmondson and Sanford (1967), Lerner and Weinstein (1967), and Greenup and Blazevic (1971), using the MIC technique, reported similar results.

Nitrofurantoin

Thirteen per cent of the strains were sensitive to nitrofurantoin. Edmondson and Sanford (1967) reported that nitrofurantoin is ineffective at low concentrations (2.5 ug/ml) but highly effective at 100 ug/ml.

Gentamycin

All of the strains were sensitive to gentamycin. These findings agree with those of other investigators. Edmondson and Sanford (1967) and Greenup and Blazevic (1971) using the MIC technique and concentration levels of 10 ug/ml and 12.5 ug/ml, respectively reported 100% susceptibility. Greenup and Blazevic (1971) also reported 100% susceptibility with the B-K method.

Kanamycin

All of the strains were sensitive to kanamycin. Edmondson and Sanford (1967) reported 100% inhibition with the MIC technique and a concentration level of 20 ug/ml.

Streptomycin

Eighty per cent of the strains were sensitive to streptomycin. Other investigators differed greatly in their findings. Edmondson and Sanford (1967) reported 28% susceptibility, and Greenup and Blazevic (1971) 100%,

both using the MIC technique and concentrations levels of 20 ug/ml and 12.5 ug/ml, respectively. As pointed out earlier, reaction to streptomycin is probably a reflection of the extent of the use of this antibiotic in the community.

Tetracycline

Seventy three per cent of the strains were sensitive to tetracycline. Other investigators have reported a wide range of susceptibilities. Edmondson and Sanford (1967) reported 56% susceptible, Greenup and Blazevic (1971) 100%, both using the MIC method and concentration levels of 20 ug/ml and 12.5 ug/ml, respectively.

Neomycin

Ninety three per cent of the strains were sensitive to neomycin.

Nalidixic acid

Eighty seven per cent of the strains were sensitive to nalidixic acid. Greenup and Blazevic (1971) reported 100% susceptibility using both the B-K method, and the MIC method and concentration level of 12.5 ug/ml.

Enterobacter agglomerans (Beijerinck) Ewing, Graham, and Fife 1972

Synonyms: *Bacterium herbicola aureum*; *B. herbicola*; *Flavobacterium trifolium*; *Pseudomonas herbicola*; *B. typhi flavum*; *Erwinia lathyri*; *E. cassavae*; *Xanthomonas herbicola*; *E. herbicola*

DISTRIBUTION

Only one isolate was obtained and the site of infection was the respiratory tract.

BIOCHEMICAL TESTS

The biochemical activities of the strain identified as *E. agglomerans* are shown in Table III. The strain was indole, Methyl Red, hydrogen sulfide and DNase negative; failed to decarboxylate lysine and ornithine or dehydrolyze arginine. Voges-Proskauer test was positive, citrate utilized and urease produced. Acid and gas were produced from glucose, and lactose, sucrose, mannitol, salicin, sorbitol, arabinose, raffinose, and rhamnose were fermented. The organism did not ferment dulcitol, adonitol and inositol, and malonate was not utilized.

ANTIBIOTIC SUSCEPTIBILITIES

The antibiotic susceptibility pattern for *E. agglomerans* is shown in Fig. VII. The results indicate resistance to ampicillin and cephalothin, and susceptibility to all others.

The species *E. agglomerans* (Herbicloa-Lathyri bacteria) has only recently been classified in the genus *Enterobacter* by Ewing and Fife (1972). No further comments or discussion will be made because of the unavailability of sufficient data on antibiotics and distribution under current or past

classification systems.

V. SERRATIA

Edwards and Ewing (1972) characterized the genus Serratia as follows: "The genus Serratia is composed of motile bacteria that conforms to the definitions of the family Enterobacteriaceae and the tribe Klebsielleae. The Voges-Proskauer reaction is positive for most strains. Lipase is produced; gelatin is liquefied rapidly. Ornithine is decarboxylated by two species (Serratia marcescens and S. liquefacans). Sodium alginate is not utilized as a sole source of carbon. When gas is formed from fermentable substrates the volumes are small (10% or less). Demonstrable extracellular deoxyribonuclease is produced. The type species is Serratia marcescens Bizio."

Of the 27 strains encountered in this study and assigned to the genus Serratia, 25 (92.6%) were identified as S. marcescens and 2 (7.4%) as S. liqueficens. The distribution of S. marcescens strains in clinical material is shown in Tables I and XI. S. marcescens is the most common and frequently encountered species, followed by S. liquefaciens; S. rubidaea is rare. S. liquefaicens and S. rubidaea, have been recovered only from the respiratory tract. The three species may be differentiated on the basis of ten biochemical tests (Table XVI).

Serratia marcescens (Bizio) Bizio, 1823

Synonyms: Bacillus marcescens; S. kilinesis; S. indica; S. plymuthica; S. piscatorum

DISTRIBUTION

Twelve of 25 strains (48%) were obtained from the respiratory tract, 24% from wounds, 16% from urine, 4% blood and 8% others (Table I). These findings are compared with those of other investigators in Table XV. All investigators reported the respiratory tract as the main site of infection, except Edmondson and Sanford (1967) who found 14.3% of their strains in the respiratory tract; Zabransky et al. (1969) reported 33.3%, Wilfert et al. (1970) 46.8% and Johnson and Ellner (1974) 44.6%. It is tempting to suggest that there has been a gradual increase in respiratory tract infections by Serratia marcescens during the past ten years but data from other clinical sites do not follow the same pattern. On the basis of present knowledge, one may conclude only that infections by Serratia marcescens are either on the increase or its occurence in increasing numbers is the result of better recognition and identification.

BIOCHEMICAL TESTS

The biochemical activities of S. marcescens strains are shown in Table IV. All S. marcescens were indole and hydrogen sulfide negative, failed to dehydrolate arginine, did not ferment lactose, dulcitol, arabinose, raffinose and rhamnose; Voges-Proskauer test was positive, lysine and ornithine decarboxylated, DNase positive and glucose, sucrose,

salicin, inositol, melibose and sorbitol were fermented, the latter without the production of gas. Over 90% of the strains gave negative Methyl Red test; malonate was not utilized. Only 16% produced urease, 20% produced prodigiosin; fermentation of xylose was variable, and gas from glucose was produced by eighty per cent of the strains.

ANTIBIOTIC SUSCEPTIBILITIES

Every strain was resistant to four or more antibiotics; the various degrees of susceptibility and resistance are shown in Figure VIII.

All strains were resistant to cephalothin and tetracycline, and sensitive to nalidixic acid. Over 90% of the strains were susceptible to gentamycin. Over 90% of the strains were resistant to nitrofurantoin. Resistance to the other antibiotics was variable.

Ampicillin

Twenty four per cent of the strains were sensitive to ampicillin. The range of reported susceptibilities extends from complete resistance to 20% susceptibility. Koch and Rose (1966) reported 64% susceptibility, Edmondson and Sanford (1967) 0.0%, Zabransky et al. (1969) 13%, Wilfert et al. (1970) 20%, and Greenup and Blazevic (1971) 8%, all using MIC techniques with concentration levels of 6.5 ug/ml, 10 ug/ml, 20 ug/ml, 20 ug/ml, and 12.5 ug/ml, respectively. Koch and Rose (1966) reported 64% susceptibility, but Greenup and Blazevic (1971) found only 4% sensitive both using the disc-diffusion method.

Carbenicillin

Sixty eight per cent of the strains were sensitive to carbenicillin. Carbenicillin, recent addition to the penicillin group, was investigated

only by Greenup and Blazevic (1971) who reported 96.5% susceptibility using MIC techniques and a concentration level of 12.5 ug/ml.

Cephalothin

All of the strains were resistant to cephalothin. This compares well with other studies. The following investigators reported 100% resistance to cephalothin using the MIC technique: Koch and Rose (1966), Ramirez (1968), Zabransky et al. (1969), Wilfert et al. (1970), and Greenup and Blazevic (1971). Greenup and Blazevic (1971) and Johnson and Ellner (1974) also found complete resistance to this antibiotic with the B-K method. Edmondson and Sanford (1967) found 3% susceptibility using the MIC technique and a concentration level of 20 ug/ml.

Polymyxin B

Sixty eight per cent of the strains were sensitive to polymyxin B. These are in disagreement with the reports of previous investigators. Edmondson and Sanford (1967) and Wilfert et al. (1970) reported 100% resistance, and Greenup and Blazevic (1971) 97%; all used the MIC technique and concentration levels of 10 ug/ml, 10 ug/ml, and 12.5 ug/ml, respectively. Johnson and Ellner (1974) found varied susceptibility to the polymyxins.

Nitrofurantoin

Ninety six per cent of the strains were resistant to nitrofurantoin, with four per cent intermediate. Edmondson and Sanford (1967) reported that nitrofurantoin was ineffective at concentrations of 2.5 ug/ml, but uniformly effective at high concentrations of 100 ug/ml.

Greenup and Blazevic (1971) reported 0.0% susceptible using the MIC technique and concentration level of 12.5 ug/ml, and 1% sensitive using the B-K method.

Tetracycline

All of the strains were resistant to tetracycline. This is similar to previous studies. Edmondson and Sanford (1967) reported complete resistance; Wilfert et al. (1970) 3% sensitive, and Greenup and Blazevic (1971) 24.5%, using the MIC technique and concentration levels of 10 ug/ml, 12.5 ug/ml, and 12.5 ug/ml, respectively. However, Greenup and Blazevic (1971) reported only 1% sensitive with the B-K method. Johnson and Ellner (1974) stated that S. marcesens varied markedly in susceptibility to tetracycline.

Gentamycin

Ninety six per cent of the strains were sensitive to gentamycin. These findings compare well with those of previous investigators. Edmondson and Sanford (1967) reported 100% susceptibility, Wilfert et al. (1970) 98.2%, and Greenup and Blazevic (1971) 100%, all used MIC techniques and concentration levels of 10 ug/ml, 6.3 ug/ml, and 12.5 ug/ml, respectively. Greenup and Blazevic (1971) also found 4% sensitivity using the B-K method.

Kanamycin

Seventy two per cent of the strains were sensitive to kanamycin. Edmondson and Sanford (1967) reported 90% inhibition, Wilfert et al. (1970) 68%, and Greenup and Blazevic (1971) 99%; all used the MIC technique and concentration levels of 20 ug/ml, 12.5 ug/ml, and 12.5

ug/ml, respectively.

Greenup and Blazevic (1971) and Johnson and Ellner (1974), using the B-K method, reported 94% and 90% susceptibility, respectively.

Streptomycin

Fifty two per cent of the strains were sensitive to streptomycin. Edmondson and Sanford (1967) reported 40% inhibition, Wilfert et al. (1970) 45%, and Greenup and Blazevic 69%, using the MIC method and concentration levels of 10 ug/ml, 20 ug/ml, and 12.5 ug/ml, respectively. Green and Blazevic (1971) also reported 67% sensitivity with the B-K method.

Neomycin

Seventy two per cent of the strains were sensitive to neomycin. Neomycin and kanamycin are aminoglycosides and as indicated earlier microorganisms often exhibit the same degree of susceptibility to both antibiotics. In this study, also, 72% of the strains were sensitive to kanamycin.

Nalidixic acid

All of the strains were sensitive to nalidixic acid, this is slightly higher than previous reports. Wilfert et al. (1970) reported 80% susceptible and Greenup and Blazevic (1971) 92.5%, both used the MIC technique and concentration levels of 3.1 ug/ml and 12.5 ug/ml respectively. Greenup and Blazevic (1971) also reported 92% sensitivity with the B-K method.

Pigment Production and Susceptibility to Antibiotics

Twenty per cent of the S. marcescens isolates were pigmented. This is in agreement with Edwards and Ewing (1972), but considerably higher than those reported by other investigators: Zabransky et al. (1969) 13.3%, Wilfert et al. (1970) 7.4%, and Johnson and Ellner (1974) 1.1%. Greenup and Blazevic (1971) reported no pigmented isolates. A comparison of antibiotic susceptibility of pigmented and non-pigmented forms shows no significant differences between the two groups. On the other hand the small number of the samples (5 pigmented and 20 non-pigmented) is not sufficient to conduct a statistically reliable analysis.

Serratia liquefaciens (Grimes and Hennerty) Bascomb, Lapage, Willcox, and Curtis, 1971

Synonyms: Enterobacter liquefaciens; Aerobacter liquefaciens; Aerobacter C

DISTRIBUTION

Both strains were isolated from the respiratory tract.

BIOCHEMICAL TESTS

The biochemical activities of S. liquefaciens are shown in Table IV. The two strains were indole, Methyl Red, hydrogen sulfide and urease negative. Voges-Proskauer was positive and citrate utilized. Lysine and ornithine were decarboxylated but arginine was not dehydrolyzed. Gas was produced from glucose; sucrose, mannitol, salicin, inositol, sorbitol and arabinose were fermented but dulcitol was not. Both strains produced DNase. No pigment was evident at 25° or 37°C. Only one of the strains fermented lactose, adonitol, raffinose and rhamnose, produced gas in inositol and sorbitol, and utilized malonate.

ANTIBIOTIC SUSCEPTIBILITIES

Susceptibility patterns are shown in Figure IX.

Both strains were resistant to ampicillin, cephalothin, and nitrofurantoin and sensitive to polymyxin B, gentamycin, kanamycin, streptomycin, neomycin and nalidixic acid. One strain was resistant to carbenicillin and the other to tetracycline.

Ampicillin

Both strains were resistant to ampicillin. Greenup and Blazevic (1971) reported 29% sensitive and Johnson and Ellner (1974) 37.5% with the B-K method. Greenup and Blazevic (1971) also reported 24% susceptible using the MIC technique and concentration level of 12.5 ug/ml.

Carbenicillin

One strain was sensitive, the other resistant to carbenicillin. Other investigators, however, found a much higher per cent of sensitivity: Greenup and Blazevic (1971) reported 94% and Johnson and Ellner (1974) 87.5%, both used the B-K method. Greenup and Blazevic (1971) also reported 94% susceptible with the MIC technique and concentration level of 12.5 ug/ml.

Cephalothin

Both strains were resistant to cephalothin. This is comparable with the findings of Johnson and Ellner (1974). Greenup and Blazevic (1971) reported 6% sensitive with the B-K method and 100% resistance with the MIC technique and concentration level of 12.5 ug/ml.

Polymyxin B

Both strains were sensitive to polymyxin B. Greenup and Blazevic (1971), however, found 100% resistance using the MIC technique and Johnson and Ellner (1974) reported 62.5% susceptibility with the B-K method.

Nitrofurantoin

Both strains were resistant to nitrofurantoin. This is similar to

the findings of Greenup and Blazevic (1971) who reported 100% resistance with the MIC technique and concentration level of 12.5 ug/ml but 12% sensitivity with the B-K method.

Tetracycline

One strain was sensitive, the other resistant to tetracycline. Greenup and Blazevic (1971) reported 86% sensitive, Johnson and Ellner (1974) 62.5%, with the B-K method, Greenup and Blazevic (1971) also reported 88% susceptibility using the MIC technique and concentration level of 12.5 ug/ml.

Gentamycin

Both strains were sensitive to gentamycin. Greenup and Blazevic (1971) reported 100% susceptibility using both the B-K method and the MIC technique with a concentration level of 12.5 ug/ml.

Kanamycin

Both strains were sensitive to kanamycin. Greenup and Blazevic (1971) and Johnson and Ellner (1974) reported 100% susceptibility using the B-K method. Greenup and Blazevic (1971) also reported 100% susceptibility with MIC technique and concentration level of 12.5 ug/ml.

Streptomycin

Both strains were sensitive to streptomycin. Greenup and Blazevic (1971) reported 88% susceptibility with the B-K method, and 89% with the MIC technique and a concentration level of 12.5 ug/ml.

Neomycin

Both strains were sensitive to neomycin. As indicated earlier

neomycin exhibits a degree of suceptibility similar to kanamycin. In this study, both strains were also sensitive to kanamycin.

Nalidixic acid

Both strains were sensitive to nalidixic acid. Greenup and Blazevic (1971) reported 100% susceptibility with both the B-K method and the MIC technique and a concentration level of 12.5 ug/ml.

VI. DISCUSSION

The necessity of proper identification of a disease causing agent cannot be overemphasized. Until the introduction of modern chemotherapy, members of the Klebsiella-Enterobacter-Serratia group, with the exception of K. pneumoniae, were considered of minor significance as a cause of serious infections. The last 30 years, however, have seen a major change in the epidemiologic patterns of this group and that of other Gram negative bacilli.

As far as is known no studies on this group relating to distribution, detailed biochemical characterization or antibiogram patterns have been attempted on the West Coast. Two hundred and three strains of the K-E-S group were collected, studied, and biochemically identified. At the generic level Klebsiella was found to be the most common (59.3%), followed by Enterobacter (27.5%), and Serratia (13.2%). The same pattern is seen in other studies (Table VII).

At the species level the study shows K. pneumoniae as the most common organism (58.8%), followed by E. cloacae (19.6%), S. marcescens (12.2%), and E. aerogenes (7.4%); K. ozaenae, E. agglomerans, and S. liquefaciens are rare. This pattern of distribution is also seen in other studies (Table VI). The data of Washington and Bourgeois (1969) differ somewhat because Serratia was not included in their study.

The distribution in clinical material of K. pneumoniae (Table IX) seems to be as common in urine as in the respiratory tract; this pattern is seen in the study of Klein et al. (1975), but is quite different from those conducted prior to 1970. Table IX suggests that the incidence of respiratory tract infections is increasing, such that today it is equal in incidence to that of the urinary tract, which was

clearly predominant prior to the 1970's.

Enterobacter cloacae, and E. aerogenes occur chiefly in the respiratory tract according to this study (Tables I, XIII, XIV). Eickhoff et al. (1966), and Edmondson and Sanford (1967) reported the urniary tract as the main source of their E. cloacae isolates; Eickhoff et al. (1966) reported no isolates from the respiratory tract. These two groups of investigators also identified the urinary tract as the chief site of isolation of E. aerogenes and the respiratory tract second. It is difficult to explain how geographic locations (Boston, Dallas, and Stockton) may affect distribution of organisms in clinical material.

Except for the report by Edmondson and Sanford (1967), the respiratory tract is the chief site of colonization by S. marcescens, and the urinary tract second (Table XV). Zabransky et al. (1969) found 33.3%, 26.7%, and 33.3% distribution in the respiratory tract, urine, and wounds, respectively. This ubiquitous organism found chiefly in the soil and to a lesser extent in the intestine, is probably misidentified in many laboratories as a species of Enterobacter. The belief that the majority of strains produce a red pigment is no longer true. In fact most strains a are non-pigmented. Its correct identification, in the absence of a pigment, rests solely on biochemical testing. Multiresistance of S. marcescens which was described by previous investigators (Wilfert et al., 1970; Greenup and Blazevic, 1971; Johnson and Ellner, 1974; and Darland, 1975) was also exhibited in this study.

A large number of biochemical tests were preformed in this study to ascertain proper identification. Tables II, III, and IV compare the results of these tests with those of Edwards and Ewing (1972). It is evident that where a large number of strains were available for study the

results were very similar.

It is a common practice among bacteriologists to accept a trait as a major characteristic of a species if 90% or more of the strains exhibit this trait. This assumption may also be applied, in a modified manner, to antibiotic patterns provided that antibiotic susceptibility studies are conducted under the same standardized conditions. In this study the disc diffusion test was used. Every effort was made to insure proper preparation of media, pH, inoculum size, incubation time, temperature, and stability of the antibiotic discs. Even so, minor variations between one preparation and another cannot be controlled. This may explain in part why certain organisms might have shown "intermediate" susceptibility rather than complete susceptibility or complete resistance to a certain antibiotic.

The use of antibiograms as an aid in the identification of bacteria is not new. It has been used for a number of years to identify certain anaerobes, especially members of the genera Bacteroides, Fusobacterium, Peptococcus, and Peptostreptococcus. It is currently being applied to the genus Pseudomonas. The basis for this idea is that certain genera or species are innately susceptible or resistant to certain antibiotics; for example, all members of the genus Proteus are resistant to polymyxin B, Proteus mirabilis is usually sensitive to penicillin G but other species of Proteus are not.

The application of this idea to the K-E-S group is relatively recent. It can be practical, time-saving and very helpful in identification if its use is based initially on a correlation between biochemical tests and susceptibility studies as was done in this work.

This practice is justified if limited to the geographic area where

the initial studies were made, and if correlations are repeated every few years, and updated. New antibiotics introduced into a community, extent of their use, the development of single or multiple resistance either by spontaneous mutations, induced mutation or through genetic recombination phenomena such as transformation, conjugation or transduction, play major roles in changing the pattern of susceptibilities. The interpretations that follow are then made with these facts in mind. No detailed discussions or conclusions will be made on K. ozaenae, E. agglomerans or S. liquefaciens since the number of isolates is not a representative sample. Reference is made to Figures I-IX and Table XVII.

As indicated earlier, using 90% as a guide line, one may make the conclusion that at the generic level the distinguishing antibiotic patterns are seen in ampicillin, carbenicillin, cephalothin, polymyxin B, and nitrofurantoin; the other antibiotics either showed uniform activity on all species, or the reactions of the organisms were variable. Because of possible differences in culture techniques, preparation of media, and conditions between experiments performed on different days, it is recommended that interpretation of the data be somewhat flexible (Tables XVIII and XIX). This is not an attempt to make the "data" fit the hypothesis but rather to take into account that differences in inoculum or slight loss in potency of an antibiotic disc may lead to different readings of the zones of inhibition. Table V indicates that a difference of one mm in the size of a zone may make a difference between "Susceptible" and "Intermediate" or "Intermediate" and "Resistant".

Using the composite antibiotic susceptibility table (Table XVII) and the interpretations suggested in Tables XVIII and XIX (S, SR, V, RS and R) the following antibiotic susceptibility patterns can be formulated.

(Table XVIII). The genus Klebsiella is characterized by RS, R, S, S, and V, representing ampicillin, carbenicillin, cephalothin, polymyxin B, and nitrofurantoin, respectively. The genus Enterobacter is characterized by RS, SR, R, S, and V, and the genus Serratia is characterized by V, V, R, V, and R, respectively.

Because of the predominance of K. pneumoniae and S. marcescens in their genera, there were no differences between the antibiogram patterns at the generic level from those at the species level (Table XIX). However, differences are evident between the genus Enterobacter (RS, SR, R, S, and V) and the species E. cloacae (R, SR, R, S, and V) and E. aerogenes (RS, V, R, S, and RS). Assuming that K. ozaenae, E. agglomerans, and S. liquefaciens are representative of their species, differences between the species, identified in this study, would be evident (Table XIX). Tables XVIII and XIX suggest distinct differences in the antibiotic susceptibility patterns, which are indicative of the genus and/or species.

VII. SUMMARY

Two hundred and three strains of the K-E-S group isolated from various clinical material from in-and out-patients at Dameron Hospital, Stockton, Calif. between January 1974 and February 1976 were investigated. The organisms were identified as follows: Klebsiella pneumoniae 120 strains (58.8%), K. ozaenae 1 strain (0.5%), Enterobacter cloacae 39 strains (19.6%), E. aerogenes 15 strains (7.4%), E. agglomerans 1 strain (0.5%), Serratia marcescens 25 strains (12.2%) and S. liquefaciens 2 strains (1.0%). Each of the species was investigated with reference to distribution in clinical material, biochemical activity, and antibiotic susceptibility. Comparisons between results obtained in this study and those in selected studies from other geographic locations in the United States were compared with respect to distribution in clinical material and antibiotic susceptibility patterns. Biochemical results were compared with those of Edwards and Ewing (1972). Antibiotic susceptibility patterns of indole-positive and indole-negative strains of K. pneumoniae were compared, and the conclusion made that the indole-positive group shows greater susceptibility to nitrofurantoin, tetracycline, and streptomycin. This is in agreement with other investigators, but the conclusion is made that additional study on a larger sample should be made to resolve the differences in results to the other antibiotics.

Comparisons were made between the antibiotic susceptibility of pigmented and non-pigmented strains of Serratia marcescens. Only minor differences were evident, but the small sample size prohibits statistical analysis.

Using a coding system of S(90-100% susceptible), SR (80-89%

susceptible), V(21-79% susceptible), RS(11-20% susceptible), and R(1-10% susceptible) as a "formula", an antibiogram pattern is suggested to describe the species encountered in this study.

Table I

Distribution of the K-E-S Group in Clinical Material

Species (number of strains)	Per cent of Total	Respiratory Tract	Urine	Wound	Stool	Blood	Other
K. pneumoniae (120)	58.8%	33.9% (40)	34.8% (42)	15.0% (18)	5.1% (6)	2.6% (3)	9.4% (11) [a]
K. ozaenae (1)	0.5%	100.0% (1)	-	-	-	-	-
E. cloacae (39)	19.6%	51.3% (20)	10.3% (4)	30.8% (12)	2.5% (1)	-	5.1% (2) [b]
E. aerogenes (15)	7.4%	53.3% (8)	26.7% (4)	20.0% (3)	-	-	-
E. agglomerans (1)	0.5%	100.0% (1)	-	-	-	-	-
S. marcescens (25)	12.2%	48.0% (12)	16.0% (4)	24.0% (6)	-	4.0% (1)	8.0% (2) [c]
S. liquefaciens (2)	1.0%	100.0%	-	-	-	-	-
Total (203)	100.0%	41.7% (84)	26.5% (54)	19.1% (39)	3.4% (7)	2.0% (4)	7.3% (15)

a = cervico-vaginal (4), eyes (2), bile (1), stomach (1), chest fluid (1) pleural cavity (1), and intubation tube (1)
b = ear (1); thumb (1)
c = stomach (1); lochia (1)

Table II*

Summary of the Biochemical Reactions Given by Klebsiella

Test or substrate	K. pneumoniae		K. ozaenae	
	%+ (120)	%+ (705)*	%+ (1)	%+ (117)*
Indole	5.8	(6.8)	0.0	(0.0)
Methyl Red	11.7	(11.3)	100.0	(99.1)
Voges-Proskauer	91.7	(93.7)	0.0	(0.0)
Citrate	98.3	(96.8)	0.0	(62.9)
Hydrogen sulfide	0.0	(0.0)	0.0	(0.0)
Urease	97.5	(95.4)	100.0	(19.8)
Motility	0.0	(0.0)	0.0	(0.0)
Lysine decarboxylase	95.8	(97.2)	100.0	(48.0)
Arginine dehydrolase	1.6	(0.6)	0.0	(6.0)
Ornithine decarboxylase	0.0	(0.0)	0.0	(4.0)
Glucose				
acid	100.0	(100.0)	100.0	(100.0)
gas	91.7	(96.0)	100.0	(66.0)
Lactose	100.0	(98.7)	0.0	(24.1)
Sucrose	100.0	(99.3)	0.0	(16.3)
Mannitol	100.0	(100.0)	100.0	(100.0)
Dulcitol	33.3	(33.0)	0.0	(0.0)
Salicin	97.5	(99.7)	100.0	(100.0)
Adonitol	(90.8)	(89.0)	100.0	(100.0)
Inositol				
acid	97.5	(97.2)	100.0	(80.2)
gas	87.5	(93.9)	100.0	(36.0)
Sorbitol	100.0	(99.4)	100.0	(88.0)

Table II* Cont.

Test or substrate	K. pneumoniae		K. ozaenae	
	%+ (120)	%+ (705)*	%+ (1)	%+ (117)*
Arabinose	100.0	(99.9)	100.0	(100.0)
Raffinose	100.0	(99.7)	100.0	(90.0)
Rhamnose	100.0	(99.3)	100.0	(68.0)
Xylose	NT	(99.9)	NT	(98.0)
Malonate	91.7	(92.5)	0.0	(4.0)
DNase	0.0	(0.0)	0.0	(0.0)
Pigment	0.0	(0.0)	0.0	(0.0)

*Figures in parentheses were obtained from Edwards and Ewing, 1972
NT = Not Tested

Table III

Summary of the Biochemical Reactions Given by

Enterobacter

Test or substrate	E. cloacae*		E. aerogenes*		E. agglomerans#	
	%+ (39)	%+ (222)*	%+ (15)	%+ (154)*	%+(1)	%+(117)#
Indole	0.0	(0.0)	20.0	(0.8)	0.0	(37.2)
Methyl Red	2.6	(3.3)	6.7	(1.6)	0.0	(42.5)
Voges-Proskauer	100.0	(100.0)	93.3	(100.0)	100.00	(57.5)
Citrate	100.0	(98.9)	100.0	(92.6)	100.00	(68.1)
Hydrogen sulfide	0.0	(0.0)	0.0	(0.0)	0.0	(0.0)
Urease	72.5	(74.6)	20.0	(5.0)	100.0	(43.5)
Motility	94.9	(92.4)	73.3	(91.7)	0.0	(88.9)
Lysine decarboxylase	0.0	(0.0)	100.0	(98.7)	0.0	(0.0)
Arginine dehydrolase	97.4	(92.4)	0.0	(0.0)	0.0	(0.0)
Ornithine decarboxylase	100.0	(93.7)	100.0	(98.7)	0.0	(0.0)
Glucose acid	100.0	(100.0)	100.0	(100.0)	100.0	(100.0)
gas	100.0	(99.3)	100.0	(100.0)	100.0	(100.0)
Lactose	76.9	(76.3)	100.0	(98.8)	100.0	(96.5)
Sucrose	100.0	(94.1)	100.0	(100.0)	100.0	(72.5)
Mannitol	94.9	(99.8)	100.0	(100.0)	100.0	(100.0)
Dulcitol	15.4	(15.2)	33.3	(4.1)	0.0	(53.1)
Salicin	74.4	(69.1)	100.0	(99.2)	100.0	(98.2)
Adonitol	30.8	(22.2)	100.0	(98.7)	0.0	(21.2)
Inositol acid	17.9	(13.0)	100.0	(100.0)	0.0	(9.8)
gas	0.0	(0.0)	100.0	(100.0)	0.0	-

Table III Cont.

Test or substrate	*E. cloacae*		*E. aerogenes**		*E. agglomerans*#	
	%+ (39)	%+ (222)*	%+ (15)	%+ (154)*	%+ (1)	%+(117)#
Sorbitol	94.9	(90.4)	100.0	(100.0)	100.0	(60.2)
Arabinose	100.0	(99.4)	100.0	(100.0)	100.0	(98.2)
Raffinose	100.0	(90.4)	100.0	(96.7)	100.0	(65.7)
Rhamnose	84.6	(89.8)	100.0	(99.2)	100.0	(99.0)
Xylose	-	(98.5)	-	(100.0)	-	(97.3)
Malonate	84.6	(80.5)	100.0	(74.7)	0.0	(66.1)
DNase	0.0	(0.0)	0.0	(0.0)	0.0	(0.0)
Pigment	0.0	(0.0)	0.0	(0.0)	0.0	(51.3)

*Figures in parenthesis were obtained from Edwards and Ewing, 1972
#Figures in parenthesis were obtained from Ewing and Fife, 1972

Table IV

Summary of the Biochemical Reactions Given by

Serratia

Test or substrate	S. marcescens*		S. liquefaciens#	
	% + (25)	% + (922)*	% + (2)	% + (117)#
Indole	0.0	(0.1)	0.0	(1.8)
Methyl Red	8.0	(18.5)	0.0	(64.2)
Voges-Proskauer	100.0	(98.7)	100.0	(49.5)
Citrate	96.0	(97.6)	100.0	(93.6)
Hydrogen sulfide	0.0	(0.0)	0.0	(0.0)
Urease	16.0	(39.7)	0.0	(3.7)
Motility	92.0	(95.0)	100.0	(92.7)
Lysine decarboxylase	100.0	(99.6)	100.0	(64.2)
Arginine dehydrolase	0.0	(0.0)	0.0	(0.0)
Ornithine decarboxylase	100.0	(99.6)	100.0	(100.0)
Glucose acid	100.0	(100.0)	100.0	(100.0)
gas	80.0	(52.6)	100.0	(72.5)
Lactose	4.0	(1.3)	50.0	(15.6)
Sucrose	100.0	(99.4)	100.0	(98.2)
Mannitol	92.0	(100.0)	100.0	(100.0)
Dulcitol	0.0	(0.0)	0.0	(0.0)
Salicin	100.0	(95.5)	100.0	(96.3)
Adonitol	92.0	(46.5)	50.0	(8.3)
Inositol acid	100.0	(77.3)	100.0	(64.2)
gas	0.0	-	50.0	(14.6)
Sorbitol	100.0	(99.1)	100.0	(97.3)

Table IV Cont.

Test or substrate	S. marcescenes*		S. liquefaciens#	
	% + (25)	% + (922)*	% + (2)	% + (117)#
Arabinose	0.0	(0.0)	100.0	(97.3)
Raffinose	0.0	(1.2)	50.0	(90.8)
Rhamnose	0.0	(0.0)	50.0	(16.5)
Xylose	28.0	(24.0)	100.0	(98.0)
Malonate	4.0	(1.6)	50.0	(0.9)
DNase	100.0	(100.0)	100.0	(100.0)
Pigment	20.0	(20.9)	0.0	(0.0)

*Figures in parentheses were obtained from Edwards and Ewing, 1972
#Figures in parentheses were obtained from Ewing et al., 1973

Table V

Zone-size Interpretative Chart for

Enterobacteriaceae, Kirby-Bauer Method

Antibiotic or Chemotherapeutic agent	Disc Potency	Inhibition Zone Diameter, mm		
		Resistant	Intermediate	Sensitive
Ampicillin	10 ug.	11 or less	12-13	14 or more
Carbenicillin	50 ug.	17 or less	18-22	23 or more
Cephalothin	30 ug.	14 or less	15-17	18 or more
Gentamycin	10 ug.	12 or less	13-14	15 or more
Kanamycin	30 ug.	13 or less	14-17	18 or more
Nalidixic acid	30 ug.	13 or less	14-18	19 or more
Neomycin	30 ug.	12 or less	13-16	17 or more
Nitrofurantoin	300 ug.	14 or less	15-18	19 or more
Polymyxin B	300 U.	8 or less	9-11	12 or more
Streptomycin	10 ug.	10 or less	12-14	15 or more
Tetracycline	30 ug.	14 or less	15-18	19 or more

Table VI

Comparison of Distribution of Species of the K-E-S Group Encountered in this Study with selected studies from other parts of the Unites States

Investigator Year (# of strains) Location	*K. pneumoniae*	*K. ozoenae*	*E. cloacae*	*E. aerogenes*	*E. agglomerans*	*S. marcescens*	*S. (E.) liquefaciens*
This Study 1976 (203) Stockton, Calif.	58.8%	0.5%	19.6%	7.4%	0.5%	12.2%	1.0%
Washington and Bourgeois 1969 (125) Rochester, Minn.	64.0%	-	22.4%	13.6%	-	-	-
Edmondson and Sanford 1967 (184) Dallas, Texas	66.7%	-	15.5%	9.5%	-	8.3%	-
Eickhoff et al. 1966 (306) Boston, Mass.	84.0%	-	10.0%	5.0%	-	1.0%	-

Table VII

Comparison of Distribution of the Genera of the K-E-S Group Encountered in this Study with selected studies from other parts of the United States

Investigator Year (# of strains) Location	Klebsiella	Entrobacter	Serratia
This Study 1976 (203) Stockton, CA	59.3%	27.5%	13.2%
Zabransky et al. 1969 (329) Rochester, Minn.	67.5%	28.1%	4.5%
Russell 1969 (478) Syracuse, N.Y.	66.5%	33.5%	-
Ramirez 1968 (67) Atlanta, Ga.	56%	32%	12%

Table VIII

Tests of Value in Differentiation of the Three Species of *Klebsiella*

Percent Positive*

Test or substrate	*K. pneumoniae*	*K. ozaenae*	*K. rhinoschleromatis*
Urease	94.5	19.3	0.0
Methyl Red	13.3	99.1	100.0
Voges-Proskauer	91.1	0.0	0.0
Citrate (Simmons')	97.7	62.9	0.0
Organic acids:			
citrate	64.4	18.0	0.0
D-tartrate	67.1	36.0	0.0
Malonate	92.5	4.0	95.5
Mucate	92.8	24.0	0.0
Lysine decarboxylase	97.2	48.0	0.0
Gas from glucose	96.5	66.0	0.0
Lactose	99.6	94.8	72.8
Dulcitol	31.5	0.0	0.0

*Edwards and Ewing, 1972

Table IX

Distribution of *K. pneumoniae* in Clinical Material

Investigator Year, (# of strains) Location	Respiratory Tract	Urine	Wound	Stool	Blood	Others
This Study 1976 (120) Stockton, Calif.	33.9% (40)	34.8% (42)	15.0% (18)	5.1% (6)	2.6% (3)	9.4% (11)
Klein et al. 1975 (250) Minneapolis, Minn.	34.4% (86)	35.6% (89)	12.8% (32)	4.8% (12)	2.8% (7)	9.6% (23)
Zabransky et al. 1969 (222) Rochester, Minn.	26.1% (58)	36.5% (81)	18.5% (41)	-	12.6% (28)	6.3% (14)
Edmondson and Sanford 1969 (112) Dallas, Texas	19.6% (22)	62.5% (70)	3.6% (4)	-	9.8% (11)	4.5% (5)
Lerner and Weinstein 1967 (84) Boston, Mass.	32.0% (27)	42.0% (36)	18.8% (15)	2.4% (2)	2.4% (2)	2.4% (2)
Eickhoff et al. 1966 (257) Boston, Mass.	8.6% (22)	59.3% (153)	2.0% (5)	6.2% (16)	19.8% (51)	1.6% (4)
Herrell et al. 1964 (120) Lexington, Ky.	31.7% (38)	52.5% (63)	8.3% (10)	-	4.2% (5)	3.3% (4)

Table X

Multiple Drug Resistance of Indole-positive and indole-negative *K. pneumoniae*

indole reaction	Number of strains resistant to: 3 drugs	4 drugs	5 drugs	6 drugs	7 drugs	8 drugs
Positive (7)	2 (28.6%)	2 (28.6%)	0	0	0	0
Negative (113)	34 (30.4%)	14 (12.2%)	9 (7.9%)	2 (1.7%)	1 (0.9%)	1 (0.9%)

Table XI

Distribution of Enterobacter spp. in Clinical Material

Investigator Year, (# of strains) Location	Respiratory Tract	Urine	Wound	Stool	Blood	Others
This study 1976, (55) Stockton, Calif.	52.7% (29)	14.5% (8)	27.2% (15)	1.8% (1)	-	3.6%
Russell 1969, (160) Syracuse, N.Y.	26.9% (43)	39.4% (63)	-	-	-	33.7% (54)
Zabransky et al. 1969, (92) Rochester, Minn.	23.9% (22)	32.6% (30)	21.7% (20)	-	13.1% (12)	8.7% (8)
Lerner and Weinstein 1967, (34) Boston, Mass.	12.0% (4)	50.0% (17)	35.0% (12)	-	-	3.0% (1)

Table XII

Tests of Value in the Differentiation of the 4 Species of *Enterobacter*

	E. cloacae	*E. aerogenes*	*E. hafnia*	*E. agglomerans*
Methyl Red	3.3	1.6	35.0	44.0
Voges-Proskauer	100.0	100.0	83.6	67.5
Lysine decarboxylase	0.0	97.5	99.6	0.0
Arginine dihydrolase	96.0	0.0	46.0	0.0
Ornithine decarboxylase	96.5	95.9	98.6	0.0
Phenylalanine deaminase	0.0	0.0	0.0	27.9
Lactose	76.3	92.5	2.8	40.5
Sucrose	94.1	99.2	7.0	77.1
Salicin	69.1	99.2	11.2	63.6
Adonitol	22.2	97.5	0.0	6.7
Inositol	13.0	96.7	0.0	14.7
Sorbitol	90.4	98.3	0.0	23.9
Raffinose	90.7	96.7	3.8	24.8

*All results are from Edwards and Ewing (1972) except for *E. agglomerans* which is from Ewing and Fife (1972).

Table XIII

Distribution of *E. cloacae* in Clinical Material

Investigator Year, (# of strains) Location	Respiratory Tract	Urine	Wound	Stool	Blood	Others
This study 1976, (39) Stockton, Calif.	51.3% (20)	10.3% (4)	30.8% (12)	2.5% (1)	-	5.1% (2)
Edmondson and Sanford 1967, (26) Dallas, Texas	30.8% (8)	46.2% (12)	7.7% (2)	-	7.7% (2)	7.7% (2)
Eickhoff et al. 1966, (30) Boston, Mass.	-	40.0% (12)	3.3% (1)	3.3% (1)	46.7% (14)	6.7% (2)

Table XIV

Distribution of *E. aerogenes* in Clinical Material

Investigator Year, (# of strains) Location	Respiratory Tract	Urine	Wound	Stool	Blood	Others
This study 1976, (15) Stockton, Calif.	53.3%	26.7%	20.0%	-	-	-
Edmondson and Sanford 1967, (16) Dallas, Texas	37.5% (6)	50.0% (8)			6.3% (1)	6.3% (1)
Eickhoff et al. 1966, (16) Boston, Mass.	18.8% (3)	68.8% (11)			12.5% (2)	

Table XV

Distribution of *Serratia marcescens* in Clinical Material

Investigator Year (# of strains) Location	Respiratory Tract	Urine	Wound	Stool	Blood	Others
This study 1976 (25) Stockton, Calif.	48% (12)	16% (4)	24% (6)	-	4% (1)	8% (2)[a]
Johnson and Ellner 1974 (74) New York, N. Y.	44.6% (33)	43.2% (32)	12.2% (9)	-	-	-
Wilfert et al. 1970 (111) Boston, Mass.	46.8% (52)	34.2% (38)	11.3% (13)	1.8% (2)	-	5.4% (6)
Zabransky et al. 1969 (15) Rochester, Minn.	33.3% (5)	26.7% (4)	33.3% (5)	-	-	6.7% (1)
Edmondson and Sanford 1967 (14) Dallas, Texas	14.3% (2)	64.3% (9)	21.4% (3)	-	-	-

a - stomach (1); lochia (1)

Table XVI

Test or substrate	S. marcescens	S. liquefaciens	S. rubidaea
Ornithine decarboxylase	99.6	100.0	0.0
Lactose	1.3	15.6	100.0
Adonitol	46.5	8.3	88.0
Sorbitol			
acid	99.1	97.3	8.0
gas	0.0	57.8	0.0
Arabinose	0.0	97.3	100.0
Raffinose	1.2	90.8	96.0
Xylose	24.0	98.0	86.0
Melibose	0.0	80.3	96.0
Malonate	1.6	0.9	86.0
Glycerol			
acid	97.2	92.2	29.0
gas	0.0	39.8	0.0

Ewing et al., 1973

Table XVII

Per cent of Strains Susceptible to Antibiotics

Species (number of strains)	Ampicillin	Carbenicillin	Cephalothin	Polymyxin B	Nitrofurantoin	Tetracycline	Gentamycin	Kanamycin	Streptomycin	Neomycin	Nalidixic acid
K. pneumoniae (120)	11.7	2.7	93.0	99.2	49.0	77.0	99.2	96.7	76.0	97.5	90.0
K. ozaenae (1)	0.0	0.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
E. cloacae (39)	10.3	82.0	0.0	100.0	61.5	77.0	100.0	100.0	92.3	100.0	100.0
E. aerogenes (15)	13.3	66.7	6.7	100.0	13.3	73.3	100.0	100.0	80.0	93.3	93.3
E. agglomerans (1)	0.0	100.0	0.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
S. marcescens (25)	24.0	68.0	0.0	68.0	0.0	0.0	96.0	72.0	52.0	72.0	100.0
S. liquefaciens (2)	0.0	50.0	0.0	100.0	0.0	50.0	100.0	100.0	100.0	100.0	100.0

Table XVIII

Interpretation of the Per cent of Strains Susceptible to Antibiotics at the Generic Level

Genus	Antibiogram Pattern				
	Ampicillin	Carbenicillin	Cephalothin	Polymyxin B	Nitrofurantoin
Klebsiella	RS	R	S	S	V
Enterobacter	RS	SR	R	S	V
Serratia	V	V	R	V	R

Per cent Susceptible Interpretation

90-100% Susceptible...S
80-89% Susceptible...SR
21-79% Susceptible...V
11-20% Susceptible...RS
0-10% Susceptible...R

Table XIX

Interpretation of the Per cent of Strains Susceptible to Antibiotics

at the Species Level

Species	Antibiogram Pattern				
	Ampicillin	Carbenicillin	Cephalothin	Polymyxin B	Nitrofurantoin
Klebsiella pneumoniae	RS	R	S	S	V
K. ozaenae	R	R	S	S	S
Enterobacter cloacae	R	SR	R	S	V
E. aerogenes	RS	V	R	S	RS
E. agglomerans	R	S	R	S	S
Serratia marcescens	V	V	R	V	R
S. liquefaciens	R	V	R	S	V

Per cent Susceptible Interpretation

90-100%	Susceptible...S
80-89%	Susceptible...SR
21-79%	Susceptible...V
11-20%	Susceptible...RS
0-10%	Susceptible...R

Figure I. Antibiogram of Klebsiella pneumoniae (120 strains)

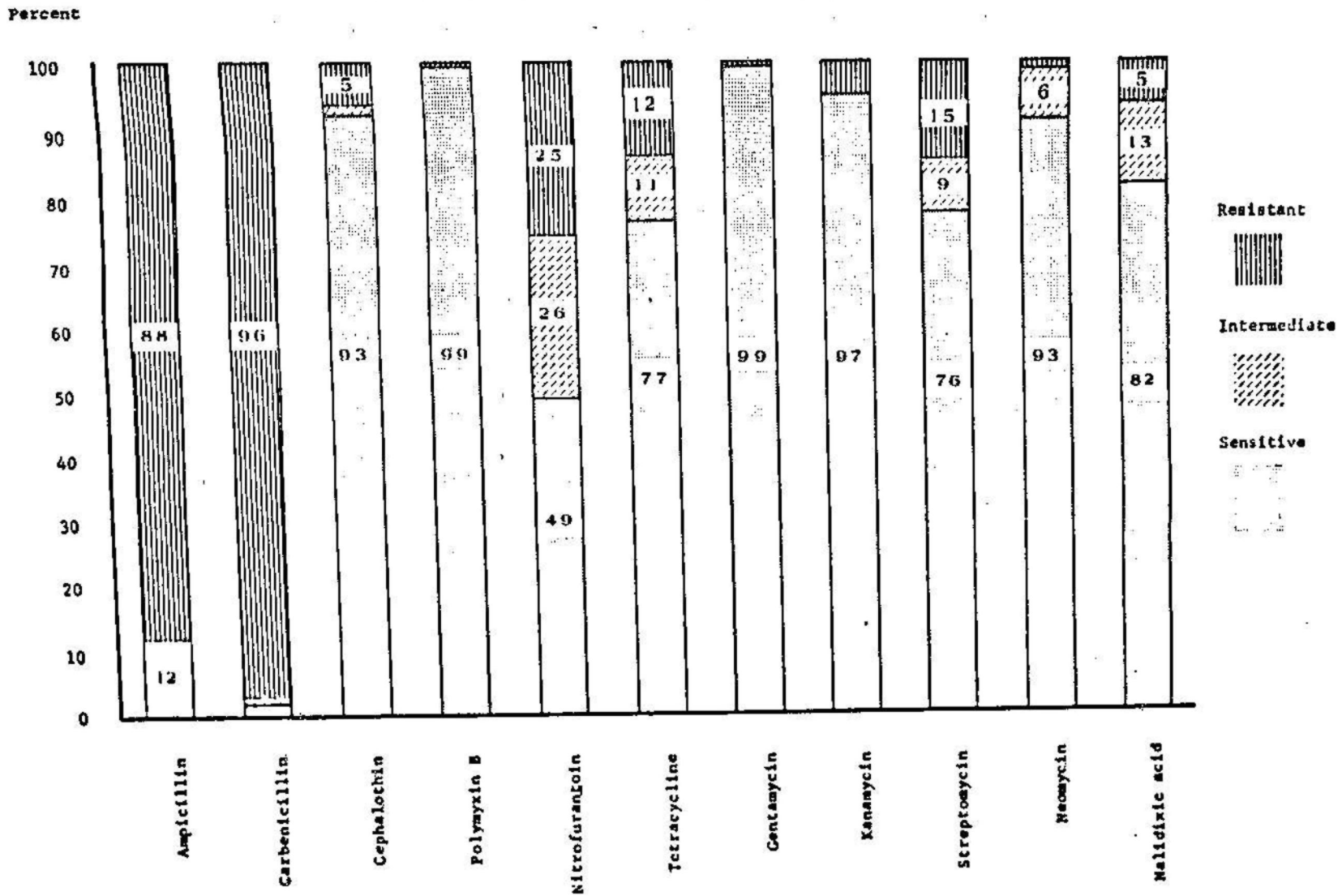


Figure II. Antibiogram of indole-positive Klebsiella pneumoniae (7 strains)

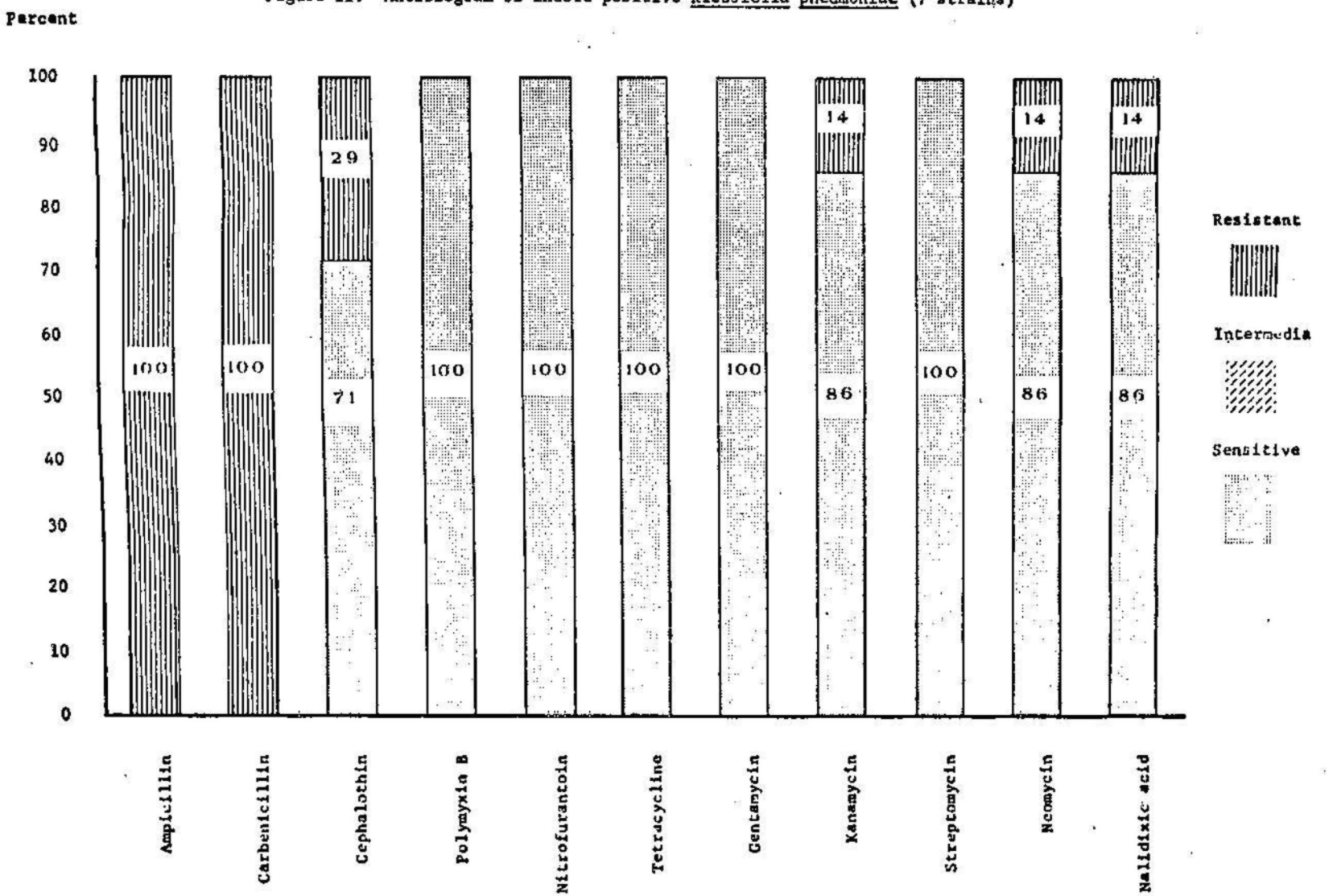


Figure III. Antibiogram of indole-negative Klebsiella pneumoniae (113 strains)

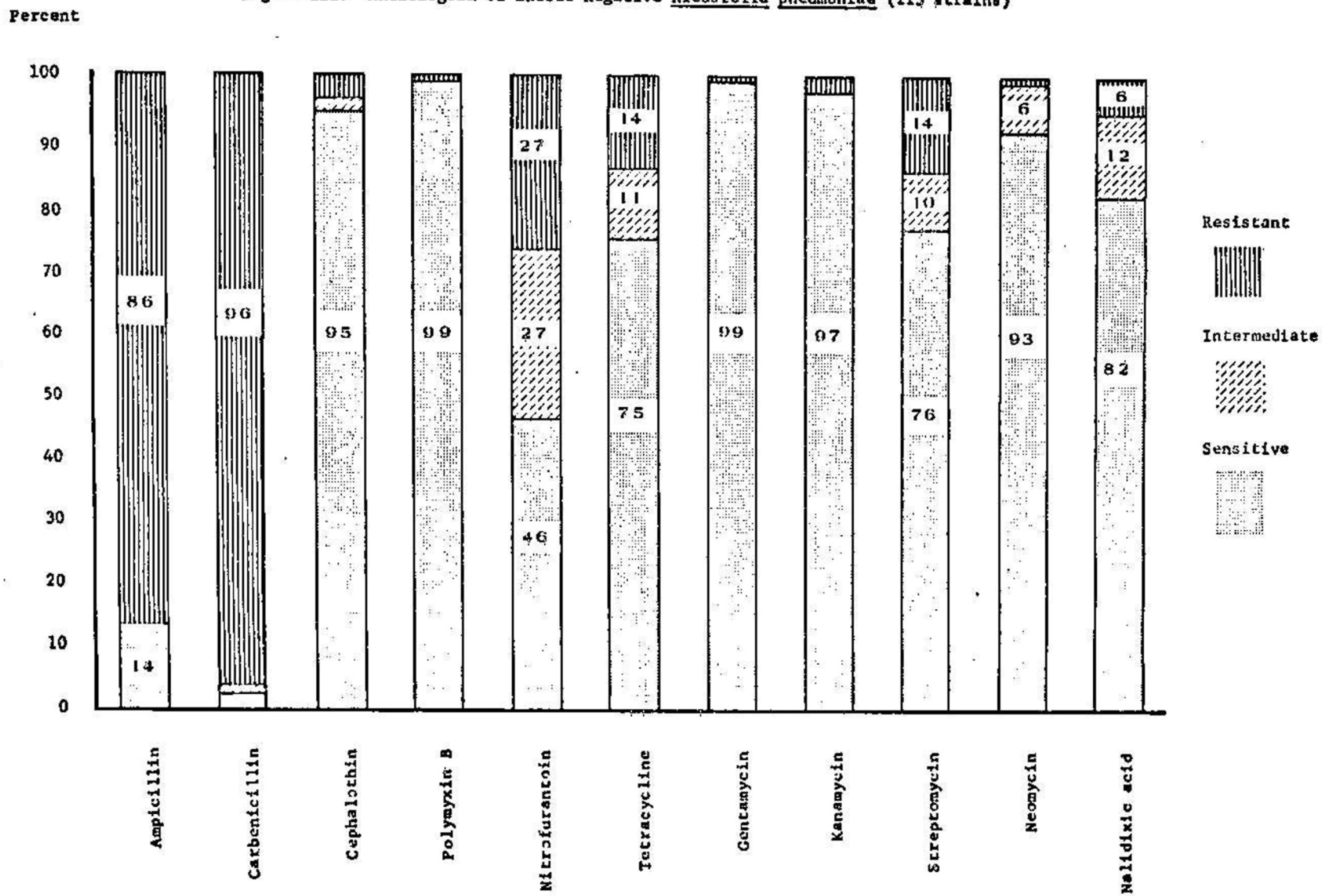


Figure IV. Antibiogram of Klebsiella ozaenae (1 strain)

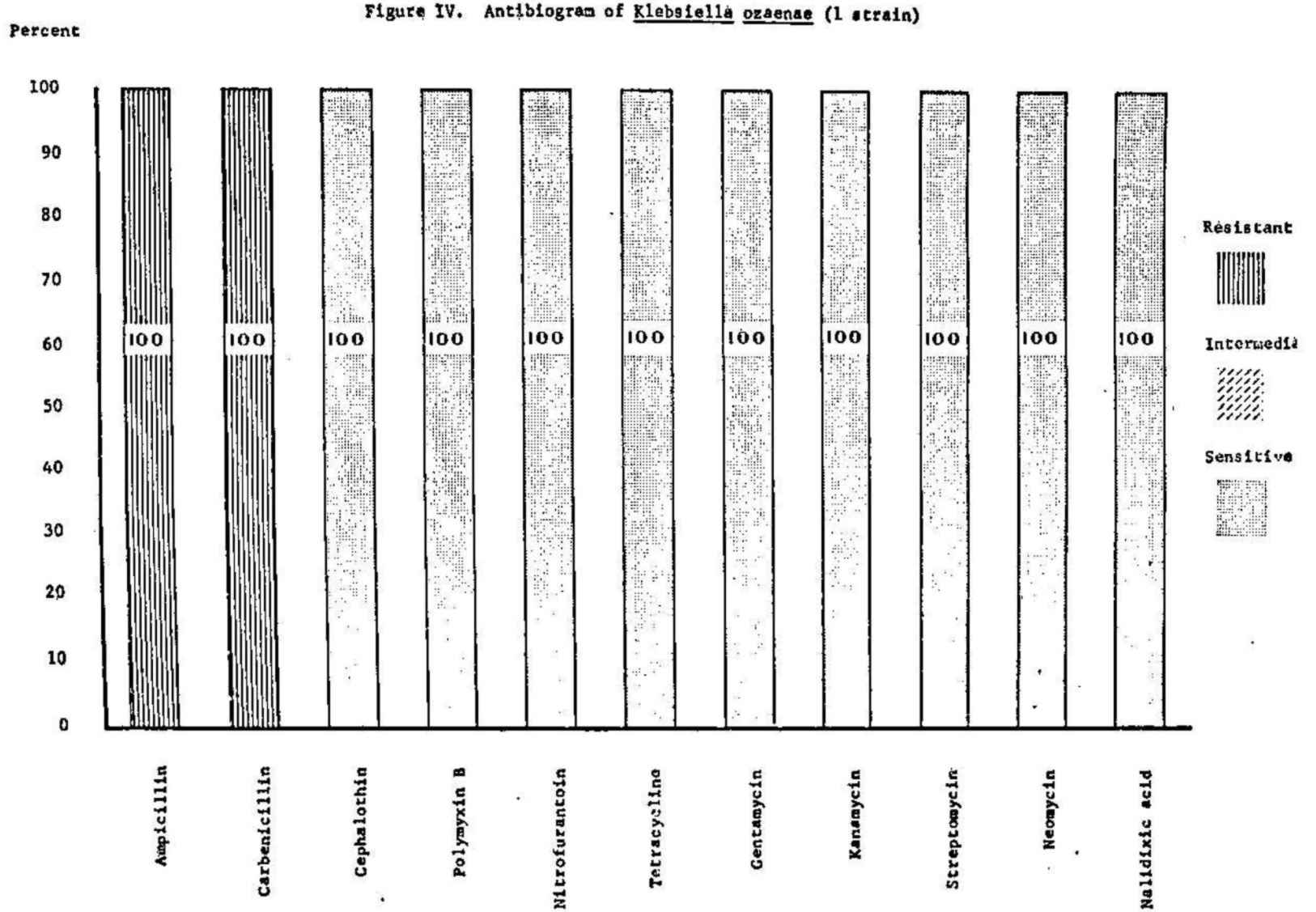


Figure V. Antibiogram of Enterobacter cloacae (39 strains)

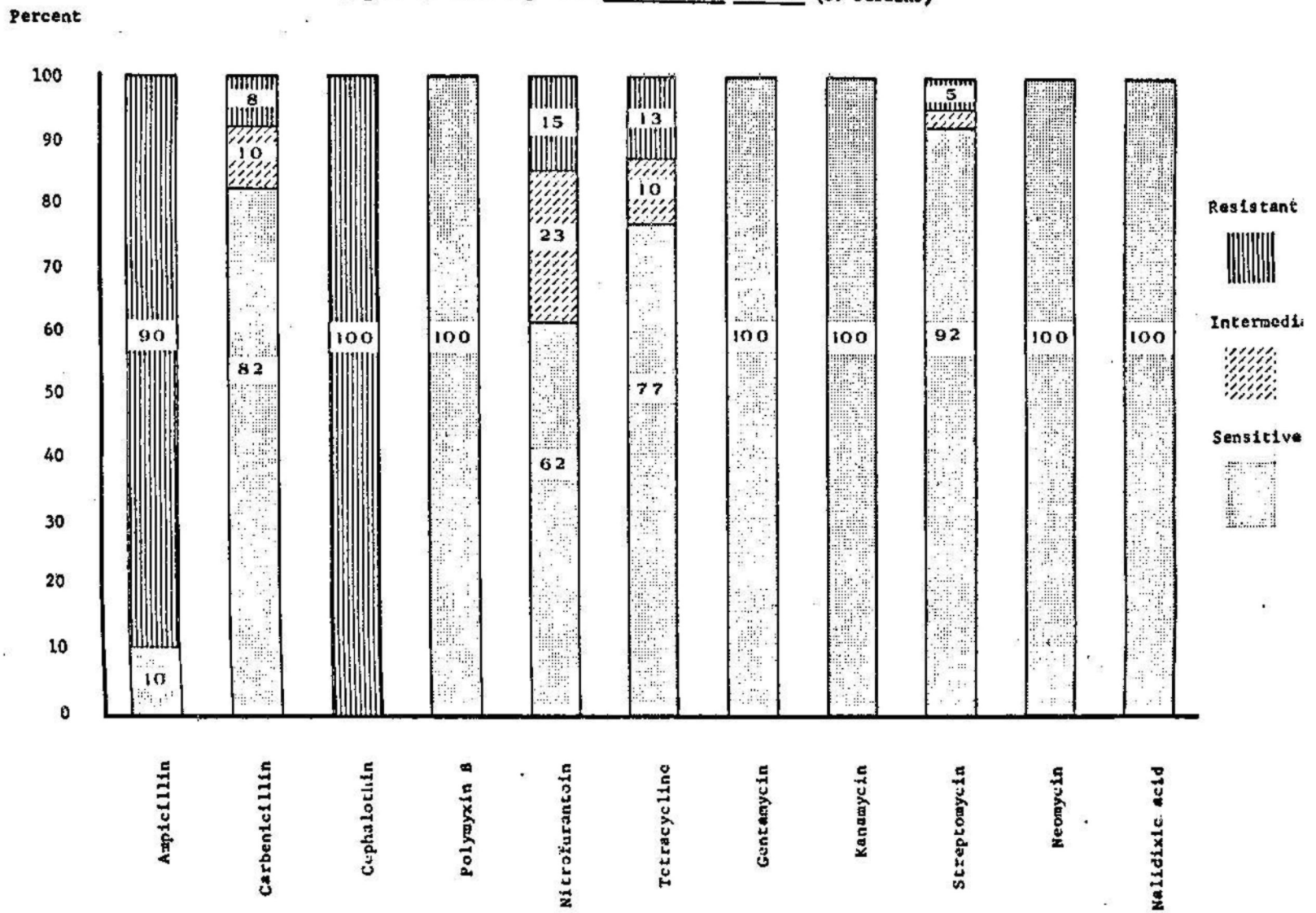


Figure VI. Antibiogram of Enterobacter aerogenes (15 strains)

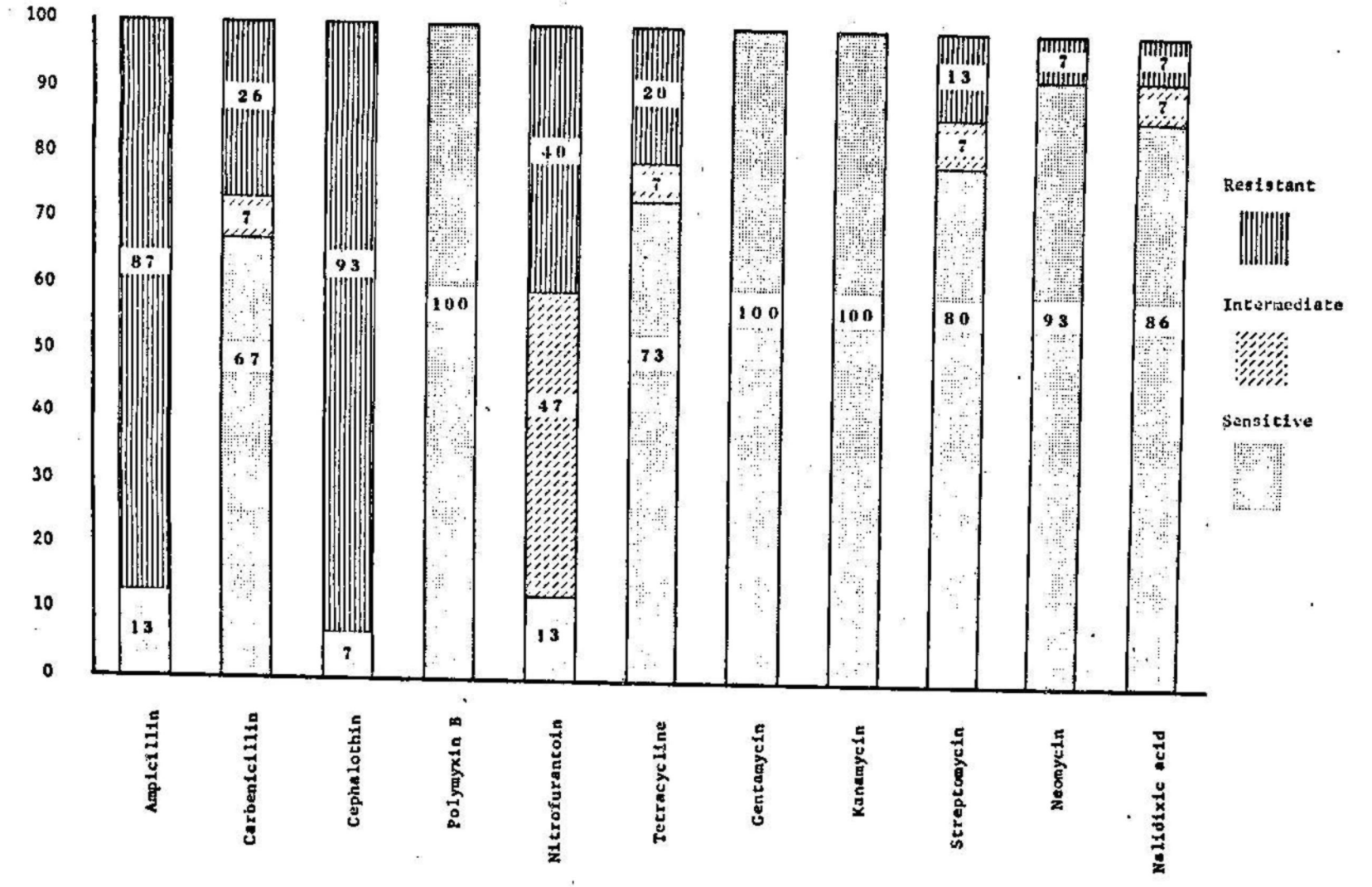


Figure VII. Antibiogram of Enterobacter agglomerans (1 strain)

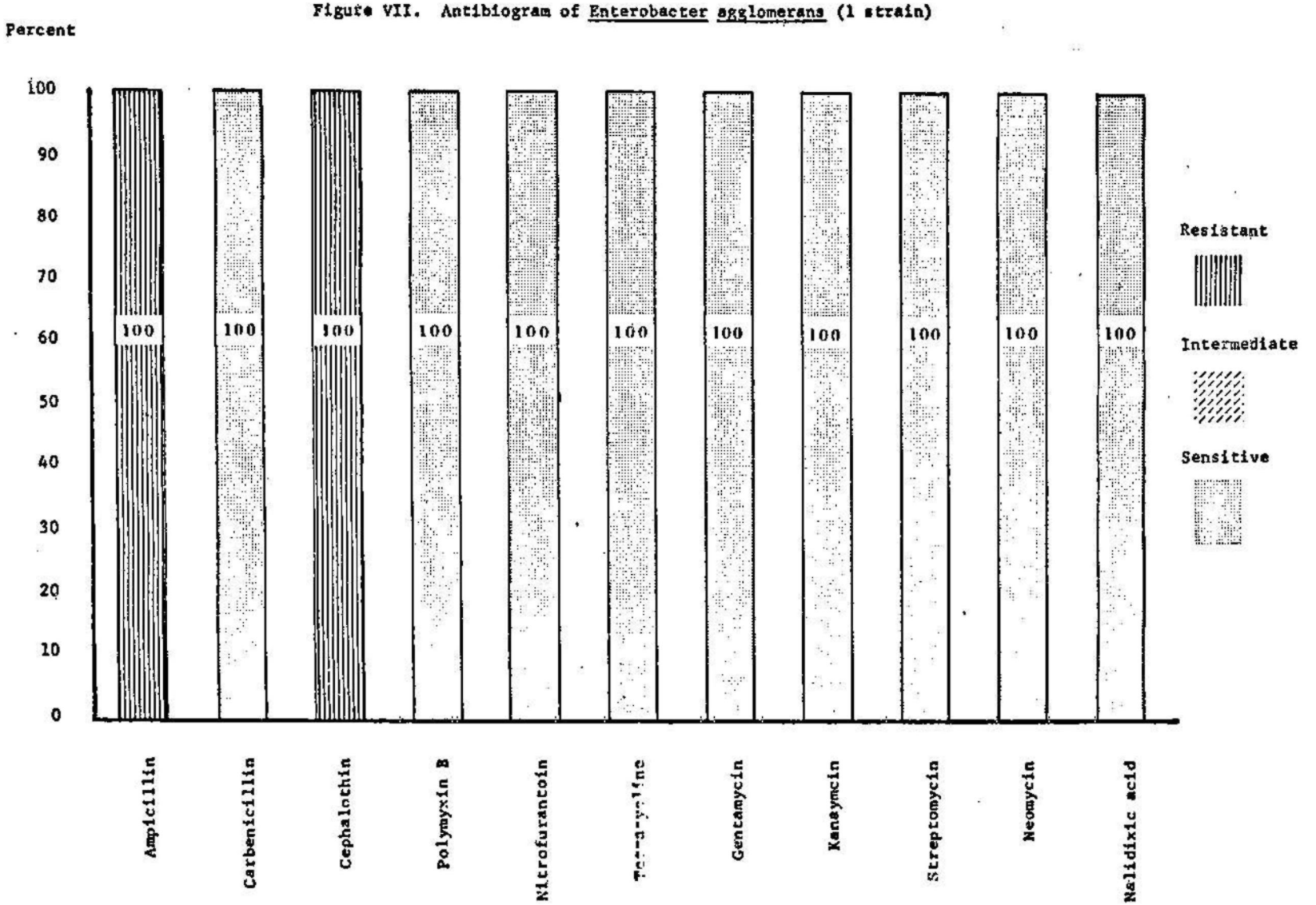


Figure VIII. Antibiogram of Serratia marcescens (25 strains)

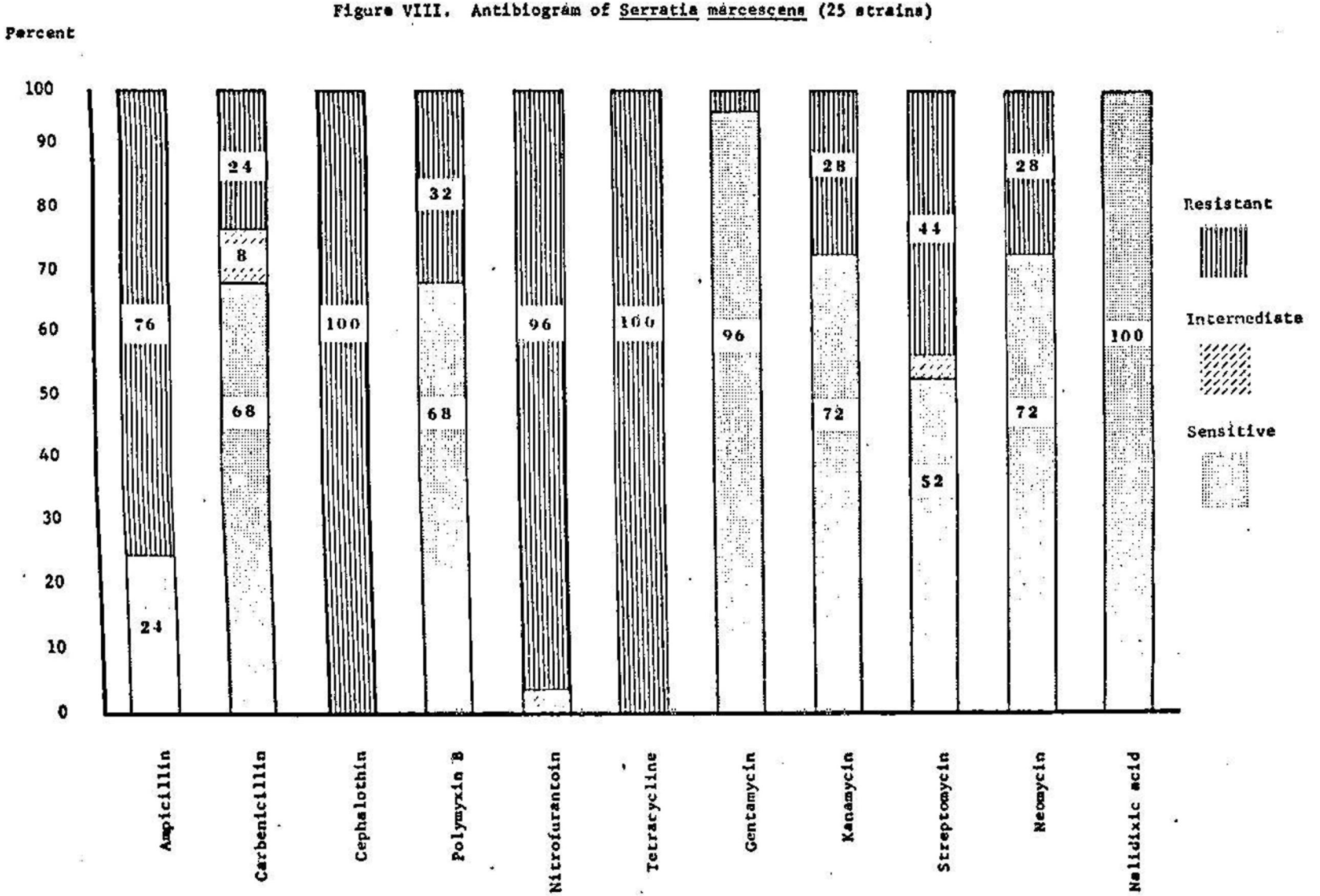
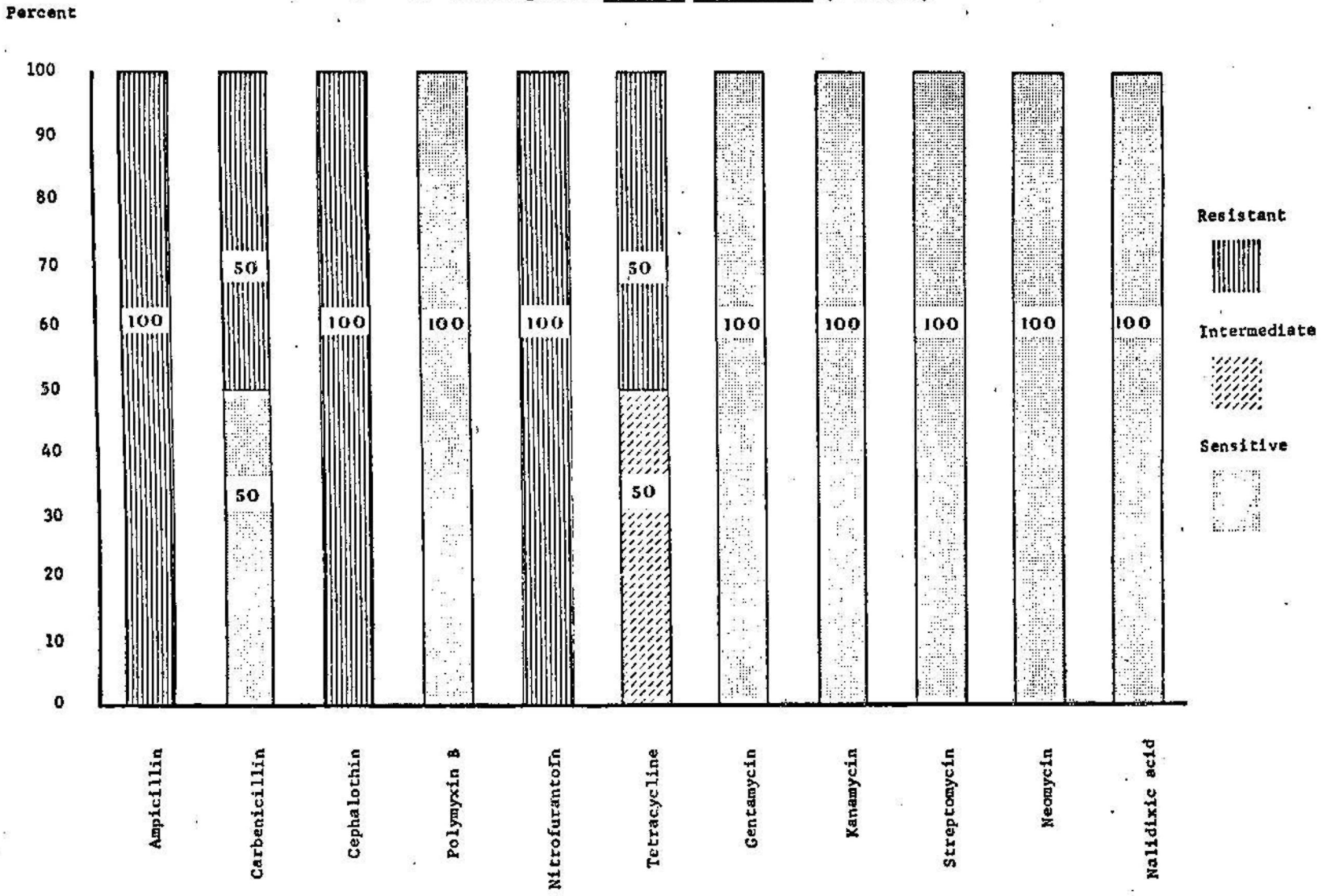


Figure IX. Antibiogram of Serratia liquefaciens (2 strains)



X. PROCEDURES

The Indole Test

Five ml aliquot of 1.5% solution of tryptone (Bacto) in distilled water is dispensed into 16 X 125 mm screw top test tubes, autoclaved at 121 C° for 15 minutes, inoculated with a drop of 24-hour nutrient broth culture and incubated at 37° C for several hours. Half ml of Kovac's reagent (Isoamyl alcohol 150 ml, paradimethylaminobenzaldehyde 10 g, and hydrochloric acid, concentrated, 50 ml) is added and tube gently shaken. The development of a deep red color at the surface within one minute is a positive test for indole production.

The Methyl Red Test

Dehydrated MR-VP Medium (Difco) in distilled water; five ml aliquot is dispensed into 16 X 125 mm screw top test tubes, autoclaved at 121° C for 15 minutes, inoculated with a drop of 24-hour nutrient broth culture and incubated at 37° C for 48 hours. Six drops of methyl red test indicator (methyl red 0.1 g, 95% ethyl alcohol 300 ml, distilled water to make 500 ml) are added and the reaction is read immediately; positive tests are bright red, weakly positive tests are red-orange and negative are yellow or orange.

Voges-Proskauer Test

Dehydrated MR-VP Medium (Difco) in distilled water; 1 ml is dispensed into each 16 X 125 mm screw top test tube, autoclaved at 121° C for 15 minutes, inoculated with a drop of 24-hour nutrient broth culture and incubated at 37° C for 48 hours. Barritt's reagents (0.4 ml of 40% aqueous KOH and 0.6 ml of 5% alpha naphthol in absolute alcohol) are added, shaking the test tube well after the addition of

each reagent. The development of a pink to red color within 30 minutes is a positive test for the production of acetymethylcarbinol (acetoin).

Citrate Test

Dehydrated Simmons' Citrated Agar (Difco) in distilled water is heated to boiling and eight ml is dispensed into 16 X 125 mm screw top test tubes, autoclaved at 121° C for 15 minutes, slanted and allowed to cool. Inoculate with a loop, using a 24-hour nutrient broth culture, streaking the surface and incubate at 37° C, and checked daily for up to seven days. A positive test is evident by growth on the slant and a change in color of the medium from green to blue.

Hydrogen Sulfide Production (TSI)

Dehydrated Triple Sugar Iron Agar (Difco) in distilled water is heated to boiling, dispensed as seven and one half ml per 16 X 125 mm screw top test tube, autoclaved at 121° C for ten minutes, slanted and allowed to cool. Inoculate with a needle using the stab-and-streak technique from a 24-hour nutrient broth culture, incubate at 37° C and check daily for up to seven days. A positive test for H_2S production is evident as a black streak on the slant or the entire medium.

The Urease Test

The Urea Agar slants are purchased from Microbiological Media, Concord, California. Inoculate with a loop, using a 24-hour nutrient broth culture, streaking the surface, incubate at 37° C, check at six hours and daily for up to seven days. A positive result is indicated by a cerise color on the slant and extending into the butt.

The Motility Test

The Motility Test Medium with 1% TCC Color Indicator is purchased from Microbiological Media, Concord, California. Inoculate with a needle, using a 24-hour nutrient broth culture, stabbing the medium, incubate at 37° C and observe daily for up to seven days. Motility is evident as a red color change into the medium from the stab line. In difficult-to-interpret cases a new motility tube is inoculated and incubated at 25° C and a hanging drop slide prepared.

Lysine and Ornithine Decarboxylase

Dehydrated Decarboxylase Medium Base (Difco) in distilled water is warmed to dissolve completely; the L-amino acid is added to make a 0.5% solution (if D-L-amino acid is used make a 1.0% solution), adjusted to pH 6.6, 5 ml aliquot is dispensed into 16 X 125 mm screw top test tubes, autoclaved at 121° C for 15 minutes, inoculate with a drop of 24-hour nutrient broth culture, layer with 4 ml sterile mineral oil, incubate at 37° C and check daily for four days. The medium first becomes yellow because of acid production from glucose; later, if decarboxylation occurs, the medium becomes alkaline (purple).

Arginine Dihydrolase

Dehydrated Decarboxylase Medium Base (Difco) in distilled water is warmed to dissolve completely; the L-amino acid is added to make a 0.5% solution (if D-L-amino acid is used make a 1.0% solution), adjusted to pH 6.6, 5 ml aliquot is dispensed into 16 X 125 mm screw top test tubes, autoclaved at 121° C for 15 minutes; inoculate with a drop of 24-hour nutrient broth culture, layer with 4 ml sterile

mineral oil, incubate at 37° C and check daily for four days. The medium first becomes yellow because of acid production from glucose; later, if decarboxylation occurs, the medium becomes alkaline (purple).

Carbohydrate Fermentations (Glucose, Lactose, Sucrose and Mannitol)

Fifteen ml aliquots of rehydrated Phenol Red Sugar (Difco) are dispensed into 20 X 150 mm test tube each with an inverted 10 X 75 mm Durham tube, and autoclaved at 121° C for ten minutes. A drop of 24-hour nutrient broth culture is introduced, incubated at 37° C and checked daily for up to seven days. A positive test for fermentation is evident as a change in color from red to yellow and gas production in the form of an "empty" space occupying ten per cent or more of the volume of the Durham tube.

Carbohydrate Fermentations (Inositol and Sorbitol)

Dehydrated Phenol Red Broth Base (Difco) and 0.7% of inositol or sorbitol in distilled water; 15 ml aliquot is dispensed into 20 X 150 mm test tubes with an inverted 10 X 75 mm Durham tube, and autoclaved at 121° C for ten minutes. A drop of 24-hour nutrient broth culture is introduced; incubate at 37° C and check daily for up to seven days. A positive test for fermentation is seen as a change in color from red to yellow; gas production is evident in the form of an "empty" space occupying ten per cent or more of the volume of the Durham tube.

Carbohydrate Fermentation (Dulcitol, Salicin, Adonitol, Arabinose, Raffinose, Melibiose and Xylose)

These Phenol Red dehydrated sugars were purchased from Key Scientific Products Company, Los Angeles, California; following the manufacturer's suggested instructions, one tablet is placed in one ml

of sterile distilled water in a 12 X 75 mm sterile disposible plastic test tube. Inoculate with a drop of a 24-hour nutrient broth culture, incubate at 37° C, check daily for up to seven days. A positive test for fermentation is seen as a change in color from red to yellow.

The Deoxyribonuclease (DNAse) Test

Dehydrated DNAse Test Agar with Methyl Green (Difco) in distilled water is heated to boiling for a few minutes, autoclaved at 121° C for 15 minutes, allowed to cool to 50-55° C and poured into 15 X 100 mm sterile Petri dishes. Inoculate with a single streak from a 24-hour nutrient broth culture, incubate at 37° C and check at 24 and 48 hours. A positive test for deoxyribonuclease (DNAse) production is indicated by a clear zone around the bacterial growth, resulting from the dissociation of highly polymerized deoxyribonucleic acid. Methyl green binds with DNA only when DNA is in a highly polymerized state.

Malonate

6 ml aliquot of dehydrated Malonate (Difco) in distilled water is dispensed into 16 X 125 mm screw top test tubes, autoclaved at 121° C for 15 minutes, inoculated with a drop of 24-hour nutrient broth culture, incubated at 37° C for 7 days and observed daily. A positive test for malonate utilization is indicated by a change in the color of the medium from green to Prussian blue.

Antibiotic Susceptibility Testing

Antibiotic susceptibility testing is conducted on Mueller-Hinton Medium (Difco) using the Bauer-Kirby standardized disc-agar diffusion method (Bauer et al. 1972). This technique attempts to standardize

procedures for susceptibility testing, the only variable being the relative susceptibility of the test organism to a specific concentration of an antimicrobial substance. The Mueller-Hinton plates are prepared by rehydrating Mueller-Hinton Medium with distilled water; bring to a boil and dispense 65 ml aliquot into 120 ml screw top glass bottles. The medium is then autoclaved at 121° C for 15 minutes, allowed to cool to 50-55° C, poured into 15 X 150 mm sterile plastic Petri dishes and stored in the refrigerator, in plastic bags until used or for a maximum of 14 days. The susceptibility testing procedure is as follows: The test organism is introduced into a nutrient broth and incubated until the density of the bacterial suspension, based upon visual observation, is approximately equal to that of a 1% $BaCl_2$ in H_2SO_4 standard (0.5 ml of a 1% $BaCl_2$ solution in 99.5 ml of 0.36 N H_2SO_4). If the bacterial suspension is too turbid it is diluted with sterile nutrient broth. The suspension is streaked on a Mueller-Hinton plate with a sterile cotton swab after excess moisture is removed by pressing the swab to the inner wall of the test tube. The plates are evenly streaked using the "three plane" method to insure equal distribution of bacterial. The surface of each plate is allowed to dry for at least five minutes but not longer than 20 minutes. Discs impregnated with antibiotics are then placed on the plates using a 12 magazine-150 mm-dispenser (Difco). The plates are incubated in an inverted position at 37° C for 18-24 hours. The diameter of each zone of inhibition is measured twice and the average compared with the standard chart from the National Committee for Clinical Laboratory Standards for interpretation of results.

The following antimicrobial drugs and concentrations (all Difco preparations) were used: ampicillin 10 ug, carbenicillin 50 ug,

cephalothin 30 ug, polymyxin B 300 U, nitrofurantoin 300 ug, gentamicin 10 ug, kanamycin 30 ug, streptomycin 10 ug, neomycin 30 ug, tetracycline 30 ug and nalidixic acid 30 ug.

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